Universidade de Aveiro Departamento de Ambiente e Ordenamento
2015

NADINE BEATRIZ LIMA ALVES

PHOTODEGRADATION OF SERTRALINE BY SOLAR RADIATION AND INFLUENCING FACTORS

ESTUDO DA FOTODEGRADAÇÃO DE SERTRALINA POR RADIAÇÃO SOLAR E ESTUDO DE FATORES DE INFLUÊNCIA

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Dissertation presented to University of Aveiro for the fulfilment of the necessary requirements to the obtainment of the degree of Master in Environmental Engineering, under the scientific supervision of Professor Maria Helena Gomes de Almeida Gonçalves Nadais, Assistant Professor from the Department of Environment and Planning of University of Aveiro, and co-supervision of Professor Valdemar Inocêncio Esteves, Assistant Professor from the Department of Chemistry from University of Aveiro and Doctor Vânia Maria Amaro Calisto, Post-Doctoral researcher from Department of Chemistry of University of Aveiro.

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Engenharia do Ambiente, realizada sob a orientação científica da Prof.ª Doutora Maria Helena Gomes de Almeida Gonçalves Nadais, Professora Auxiliar do Departamento de Ambiente e Ordenamento da Universidade de Aveiro, e co-orientação do Prof. Doutor Valdemar Inocêncio Esteves, Professor Auxiliar do Departamento de Química da Universidade de Aveiro e da Doutora Vânia Maria Amaro Calisto, Investigadora de Pós-Doutoramento do Departamento de Química da Universidade de Aveiro.

What could I say to you that would be of value, except that perhaps you seek too much, that as a result of your seeking you cannot find.
Hermann Hesse

À minha mãe, ao meu pai e ao meu irmão

o júri

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keywords

Photodegradation, photossensitizers, environment, water, pollution, pharmaceuticals, antidepressants, sertraline, high pressure liquid chromatography.

abstract

Photodegradation is considered to be one of the most important processes of elimination of pharmaceutical drugs from natural water matrices. The high consumption and discharge of these substances, in particular antidepressants, to the aquatic environment supports the need to study degradation processes.

This dissertation aimed at studying the direct and indirect photodegradation of sertraline, an antidepressant known for its persistence in the environment, and the evaluation of the influence of environmentally relevant factors in its photodegradation.

The photodegradation experiments were developed under simulated solar light and the irradiation times converted to summer sunny days (SSD), an equivalent time in natural environmental conditions. The direct photodegradation was evaluated in solutions of sertraline prepared in ultrapure water and the indirect photodegradation was studied through the addition of photosensitizers (humic substances, Fe(III), nitrates and oxygen). Further irradiation studies were perfomed in aqueous samples collected from two wastewater treatment plants, Vouga river and *Ria de Aveiro*. The samples were chemically characterized (dissolved organic carbon, nitrates and nitrites and iron determination and UV/Vis spectroscopy). The quantification of sertraline was done by HPLC-UV and photoproducts from direct photodegradation were identified by electrospray mass spectrometry.

An observed direct photodegradation rate of sertraline of 0.0062 h^{-1} was determined, corresponding to a half-life time of 111 h (equivalent to 29 SSD). A significant influence of photosensitizers was observed, the best results being achieved in irradiations of sertraline with humic acids, obtaining a half-life time of 12 h. This was attributed to the hydrophobicity of this substance and higher absortivity in the UV/Vis wavelength, which promote processes of indirect photodegradation.

The degradation of sertraline in natural samples was also enhanced comparatively to the direct photodegradation, achieving half-life times between 10 and 25h; the best results were achieved in samples from the primary treatment of a wastewater treatment plant and *Ria de Aveiro*, with half-life times of 10 and 16 h, respectively.

A total of six photoproducts formed during the direct photodegradation of sertraline were identified, three of which were not yet identified in the literature.

The main factors contributing to the degradation of sertraline were analysed but this was not fully accomplished, requiring further studies of the composition of the natural matrices and the combined influence of distinct photosensitizers during the irradiation. Nevertheless, it was concluded that the photodegradation of sertraline is greatly influenced by indirect photodegradation processes, promoted by the presence of photosensitizers.

palavras-chave

Fotodegradação, fotosensibilizadores, ambiente, água, poluição, fármacos, antidepressivos, sertralina, cromatografia líquida de alta pressão.

resumo

A fotodegradação é considerada um dos mais importantes processos de eliminação de fármacos de águas naturais. O elevado consumo e descarga destas substâncias nos meios aquáticos, em particular antidepressivos, suporta a necessidade de estudo destes processos de degradação.

Esta dissertação teve como objetivos o estudo da fotodegradação direta e indireta da sertralina, um antidepressivo conhecido pela sua persistência no ambiente, e a avaliação da influência de fatores ambientalmente relevantes na sua fotodegradação.

Os ensaios de fotodegradação foram desenvolvidos com radiação solar simulada e os tempos de irradiação convertidos a dias de sol de verão (*summer sunny days*, SSD), um tempo equivalente para condições ambientais naturais. A fotodegradação direta foi avaliada através da irradiação de sertralina em água ultrapura e, por sua vez, a fotodegradação indireta com recurso a fotossensibilizadores (substâncias húmicas, Fe(III), nitratos e oxigénio). Estudos posteriores foram realizados com amostras aquosas recolhidas de duas estações de tratamento de águas residuais (ETAR), do rio Vouga e da Ria de Aveiro. As amostras foram caracterizadas quimicamente (determinação de carbono orgânico dissolvido, de nitratos e nitritos e de ferro e espectroscopia UV/Vis). A sertralina foi quantificada recorrendo a HPLC-UV e os fotoprodutos de fotodegradação direta foram identificados por espetrometria de massa.

A taxa observada de fotodegradação direta de sertralina foi de 0.0062 h^{-1}, correspondendo a um tempo de meia vida de 111 h (equivalente a 29 SSD). Verificou-se uma influência significativa dos fotossensibilizadores, tendo-se obtido os melhores resultados em irradiações de sertralina com ácidos húmicos, com um tempo de meia vida de 12 h. Isto foi atribuído às características hidrofóbicas desta substância e à sua elevada absortividade nos comprimentos de onda da gama UV/Vis, características que promovem processos de fotodegradação indireta.

A fotodegradação da sertralina em amostras naturais também aumentou face à fotodegradação direta, tendo-se alcançado tempos de meia vida entre 10 e 25 h; os melhores resultados foram obtidos em amostras provenientes do tratamento primário de uma ETAR e da Ria de Aveiro, com tempos de meia vida de 10 e 16 h, respetivamente.

Foram identificados um total de seis produtos de fotodegradação direta de sertralina, três dos quais não foram ainda identificados na literatura.

Os principais fatores contribuintes para a fotodegradação de sertralina foram analisados mas não foram completamente determinados, sendo necessários mais estudos sobre a composição das matrizes naturais e da influência combinada de fotossensibilizadores distintos. Não obstante, concluiu-se que a fotodegradação de sertralina é fortemente influenciada por processos de fotodegradação indireta, promovidos pela fotossensibilização.

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Abbreviations

ACN	Acetonitrile
BOD	Biochemical Oxygen Demand
CIT	Citalopram
DDD	Daily Defined Doses
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DIC	Dissolved Inorganic Carbon
FA	Fulvic Acids
FLX	Fluoxetine
FVX	Fluvoxamine
HA	Humic Acids
HPLC	High-Performance Liquid Chromatography
HRT	Hydraulic Retention Time
HS	Humic Substances
IC	Inorganic Carbon
LOD	Limit of Detection
LOQ	Limit of Quantification
NHS	National Health Service
PAR	Paroxetine
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
RA	*Ria de Aveiro*
SER.HCl	Sertraline Hydrochlorated
SER	Sertraline
SRT	Solids Retention Time
SSD	Summer Sunny Day
SSRI	Selective Serotonin Reuptake Inhibitor
UV	Ultra-Violet
UV/Vis	Ultra-Violet/Visible

TC	Total Carbon
TOC	Total Organic Carbon
VR	Vouga River
WTP	Water Treatment Plant
WWTP	Wastewater Treatment Plant
XAD-4	XAD-4 fraction of humic substances

Nomenclature

A	Peak area
Abs	Absorbance
C_0	Concentration of the analyte at t_0 moment, as measured by the analytical method
$C_{t,i}$	Concentration of replicate *i* at *t* time of irradiation
$C'_{t,i}$	Corrected concentration of replicate *i* at *t* time of irradiation, in function of the concentration variation of the dark controls
$Deg_{S,t,i}$	Concentration variation of replicate *i* of irradiated samples at *t* time of irradiation, in function of C_0
$Deg_{C,t,i}$	Concentration variation of replicate *i* of dark controls at *t* time of irradiation, in function of C_0
$Deg_{C,t}$	Average of concentration variation of dark controls at *t* time of irradiation, in function of C_0
$Fot_{t,i}$	Photodegradation of replicate *i* at *t* time of irradiation
h	Peak height
k_{obs}	Observed photodegradation rate
log K_{OC}	Organic Carbon Adsorption Coefficient
log K_{OW}	Octanol-Water Partition Coefficient
n	Number of replicates
r^2	Determination coefficient
t_m	Void time
t_r	Retention time
t_s	Time in stationary phase
$t_{1/2}$	Half-life time
ε	Absorptivity
λ	Wavelength
σ	Standard deviation

Chapter 1 Introduction

1.1 Pharmaceuticals in the environment

1.1.1 Consumption of pharmaceuticals in Portugal

Back in 2010 a statistical study was developed on Mental Health in the European Union (organized by the Eurobarometer), in order to gather information on the state of mental health of the population, as well as their actions to improve it. It was demonstrated that "the use of antidepressants is highest in Portugal, where the prevalence of use is double than that of the EU average (15%)" (Eurobarometer 2010).

Infarmed, Portugal's National Authority for Medication and Health Products, sorts pharmaceutical substances by therapeutic groups and subgroups (see Figure 1.1). Antidepressants belong to the group "Central Nervous System", subgroup of "Psychodrugs". Looking further into the pharmaceuticals consumption in Portugal in 2010 (the same year as Eurobarometer's statistical study), an Infarmed study showed that the pharmacotherapeutic group "Central Nervous System" (which include, among others, antidepressants, anxiolytics, muscle relaxants and antiepiletics) had the second highest contribution to the National Health Service (NSH) ("Cardiovascular system" came in first, and "Endocrine system" in third place) (Infarmed 2010), which is in accordance with the population's perception of the state of mental health.

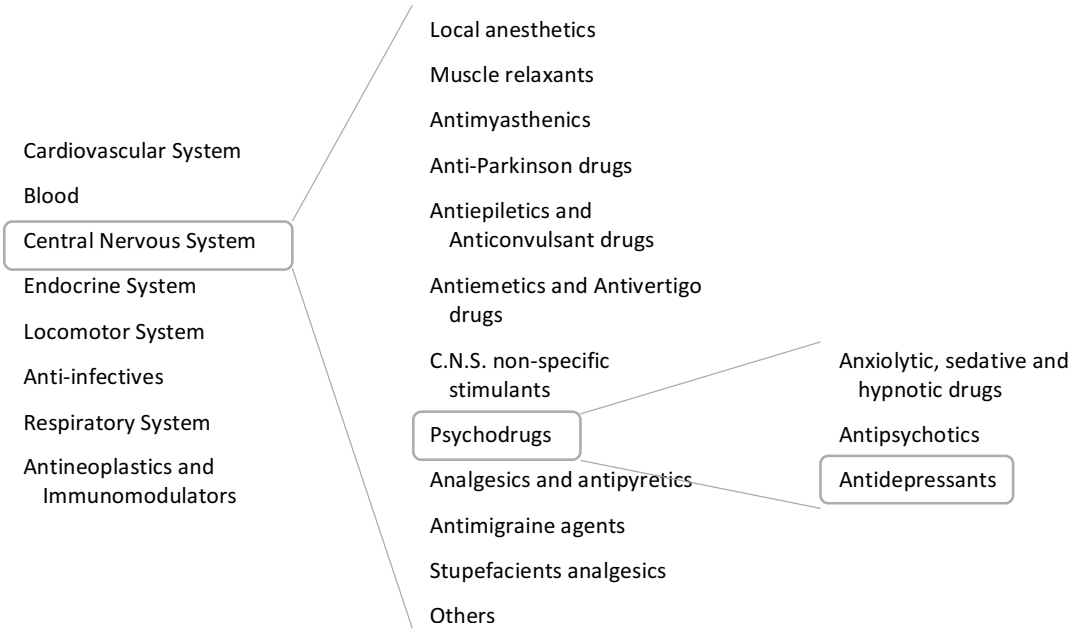

Figure 1.1 – Main therapeutic groups and subgroups of pharmaceutical drugs according to Infarmed.

The latest divulged study (Infarmed 2013) remarked that, within the top 100 active substances with the highest number of sold medicine packages in the NHS, 11 substances belong to the

"Psychodrugs" subgroup (alprazolam, an anxiolytic, being the first of this group, raking 7th). Within the antidepressants, 5 are included in this list, sertraline, venlafaxine, fluoxetine, escitalopram and trazodone, in the ranks 35, 38, 40, 55 and 58 respectively.

"Nowadays, sertraline is the market leader in [the antidepressant] subgroup", as can be seen in Figure 1.2. A decade ago, the most used antidepressant was fluoxetine, however, since sertraline started being considered a subsidized pharmaceutical, it started being more used (Furtado 2014), leading to higher consumptions of this medicinal product.

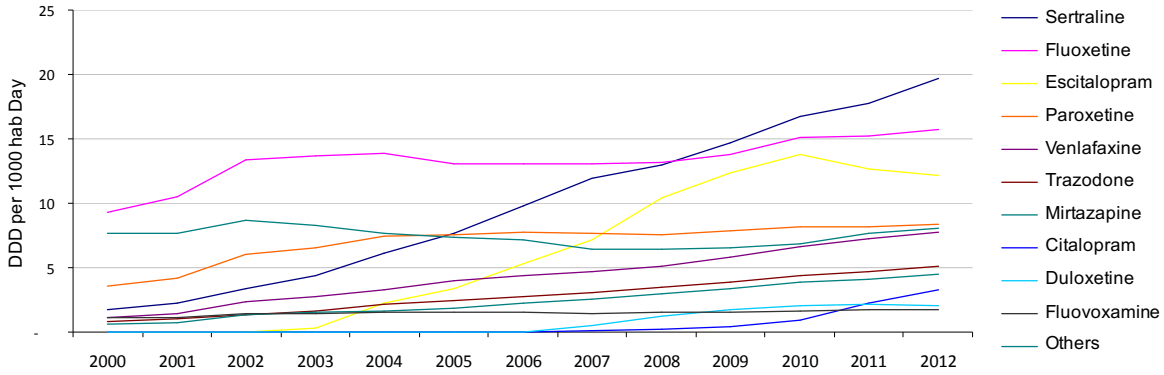

Figure 1.2 – Evolution of the use of the main active substances (antidepressants) between 2000 and 2012. DDD: Defined Daily Doses. Adapted from Furtado (2014).

Overall, the rise of the consumption of antidepressants in Portugal during the past decade is very clear. Considering the nature of these substances – which act directly in the central nervous system of an organism, this consumption growth suggests the need for thorough studies regarding the possible discharge sources of the substances into the environment, their behaviour and persistence, as well as their toxicity to the exposed organisms.

1.1.2 Sources of pharmaceuticals into the environment

When studying the main sources of discharges of a specific pollutant into the environment, one must take into consideration several factors, such as the behaviour of the pollutant itself in different matrices (aqueous *versus* soils/sediments, for instance), the natural sources of this pollutant (which is insignificant in this case, since most pharmaceutical compounds are anthropogenic), accidental discharges, point and diffuse sources, as well as the structural changes that the compound may suffer after its ingestion.

Taking these factors into consideration, several authors have done research studies on the main sources of pharmaceuticals into the environment (Kümmerer 2010; Calisto 2011; Homem 2011). Figure 1.3 summarizes some of the main sources identified by these authors and their dissipation paths. The studied sources contemplate mostly anthropogenic activities and are conditioned by the manufacture, consumption and disposal of pharmaceutical compounds.

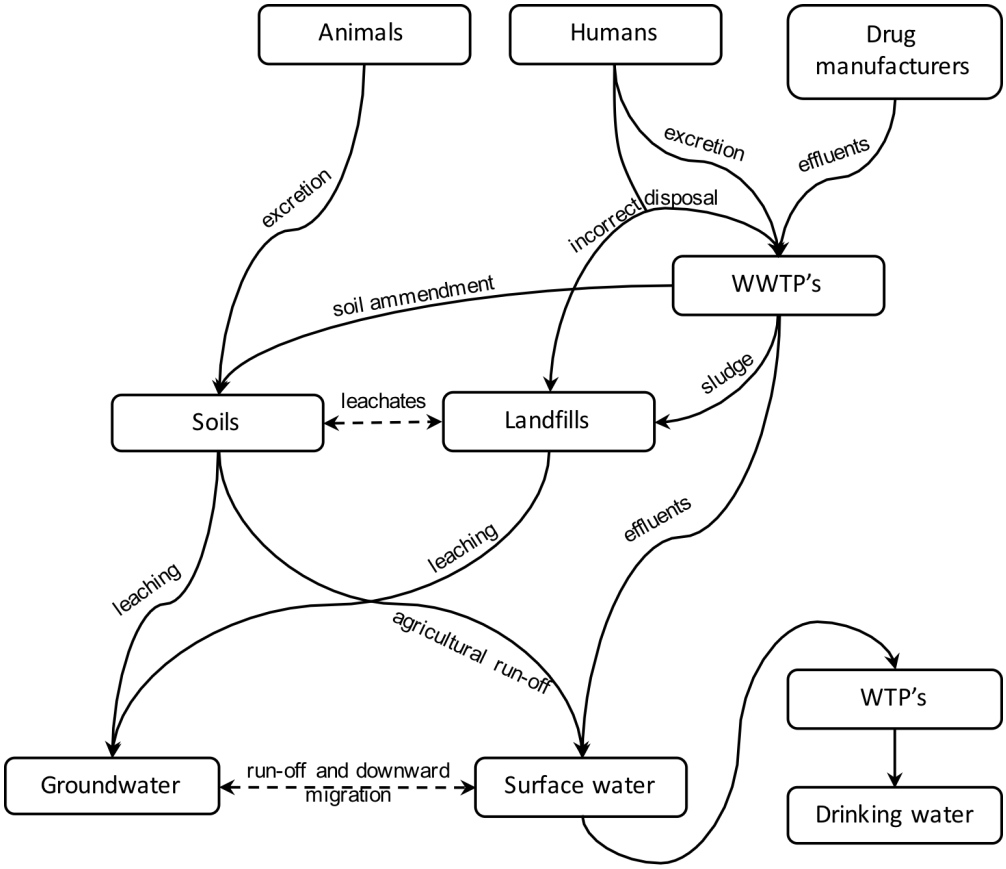

Figure 1.3 – Main sources of pharmaceuticals into the environment. WWTP: Wastewater Treatment Plant, WTP: Water Treatment Plant. Adapted from Calisto *et al.* (2011), Homem (2011); and Kümmerer (2010).

The release of pharmaceutical substances from the manufacturers has only been a subject under study during the past decade, specifically in a plant situated in an Indian city (Patancheru, Hyraderabad) that receives wastewater primarily from drug manufacturers. These effluents were reported as containing very high concentrations of at least 11 of the analysed drugs (levels above 100 μg/L) (Larsson *et al.* 2007). Additionally, a study on the USA conducted during the period of 2004-2009, concluded that pharmaceutical manufacturing industries were the source of some compounds detected downstream of the industries, and that were not found elsewhere in the stream (U.S. Geological Survey 2015a).

It has been stated that, as of 2009, there were not enough published studies to sustain the premise that these industries contribute significantly to the overall discharge of drugs to the environment (Larsson & Fick 2009). However, studies have been conducted to analyse the impact to organisms caused by these effluents (in diluted concentrations), concluding that fish and amphibians are affected in their physiological processes (Cardoso *et al.* 2014).

The consumption of pharmaceuticals concerns the ingestion of drugs by organisms, the structural changes that may happen within them and their excretion in urine and faeces. After ingestion, the fate of the drug is specific for each drug and organism, and may be either excreted as the unchanged parent compound and/or as metabolites or conjugates (Calisto 2011; Kümmerer 2010).

The main pathway of pharmaceuticals into the environment is considered the consumption and incorrect disposal of medicine, as it creates a continuous flow of pharmaceutical substances into the environment. The consumption by humans leads to a possible discharge of drugs/metabolites through the domestic wastewaters, ending up in the wastewater treatment plants (WWTP) where, depending on the level of treatments applied to the effluent and on the drug, it might be efficiently degraded or only partially degraded and consequently discharged into waterbodies. Pharmaceuticals might also adsorb onto the biosolids during treatments. Usually these biosolids are then used for soil amendment, leading to the contamination of agriculture fields, and subsequently surface water bodies through run-offs and groundwater by leaching.

The consumption by animals is usually more significant for cattle, and the excrements directly contaminate the soils, taking the same path described previously, until reaching surface water and groundwater.

In terms of the contributions of landfills, a study has shown that municipal landfills contribute to the presence of emerging compounds in the environment, having concluded that pharmaceutical compounds were detected in their leachates (Eggen *et al.* 2010). This information is crucial when we consider the amount of drugs and pharmaceutically active compounds that are incorrectly disposed of along with the common waste.

Portugal is currently taking measures, through the organization Valormed, by collecting unused medicines and the respective packages, but this relies on the participation of the population. Regardless, efforts have been made in order to avoid the discharge of unused medicines to the municipal waste flow. In 2007, the REACH (Registration, Evaluation and Authorization of Chemicals) regulation was implemented in Portugal, in order to provide a framework that allows the regulation and restriction of chemicals that can have environmental impacts during their life cycle.

1.1.3 Ecotoxicology of antidepressants

A guideline has been released for the environmental risk assessment of medicinal products for human use (EMEA 2006) which recommends tests such as growth inhibition in algae, *Daphnia* reproduction and fish early life stage toxicity (Fent 2008). However, aquatic organisms may be exposed to a series of pollutants during their entire span of life, and this parameter is not usually taken in to account when developing these standardized tests. This results in a poor ecotoxicological risk assessment.

The mode of action of a pharmaceutical is just as important as their possible side effects, as either one or the other might be reflected on the aquatic organisms, and much depends on the particular organism.

The main objective of SSRIs (Selective Serotonin Reuptake Inhibitors) is to inhibit the reuptake of serotonin, whereas other types of antidepressants' objective might be directed at the reuptake of norepinephrine or dopamine. In a human brain, SSRIs work on the synapse space between two nerve cells. Serotonin is expected to be transported from the sending cell to a receiving cell, but when this process does not occur completely, some of the serotonin is reabsorbed by the sending cell (reuptake of serotonin), causing an imbalance in the serotonin levels. This is when SSRIs come into action, by blocking its reuptake, forcing the serotonin to either be sent to the receiving cell or undergo natural breakdown by monoamine neurotransmitters. The neurotransmitter Serotonin is also found in lower vertebrates and invertebrates and acts on the immune system, mood and appetite regulation and influences the behaviour of the organism as well as its sexual functions (Fent 2008). For example, Lamichhane *et al.* (2014) found that sertraline affects small crustaceans like *Daphnia magna* and *Ceriodaphnia dubia* on their reproduction as well as their growth, inhibiting it.

Moreover, the side effects of any particular pharmaceutical drug must be considered and may have a different effect on different organisms, at different concentration ranges, which is why it is so important to study each one very thoroughly.

For some drugs, as is the case of antidepressants, the mode of action is not always fully known. Tricyclic antidepressants' mode of action is still unclear, despite its effectiveness (Ciraulo *et al.* 2011), for instance. Thus, the pharmaceutical might affect another organism through pathways that are still unclear and have unexpected results.

Despite the pursue for better biological endpoints (Park *et al.* (2012) in his search for a biomarker of exposure, for instance) mortality is still used as an assessment of ecotoxicology (Minagh *et al.* 2009; Schultz *et al.* 2011).

When possible, the ecotoxicology of a pharmaceutical substance is determined according to traditional standard tests from established data, using organisms like algae, zooplankton and fish. Whenever this is not feasible, there is the possibility to estimate the ecotoxicological effects through models like ECOSAR (Ecological Structure Activity Relationships predictive model) (Fent 2008).

Environmental risk assessments often resort to "predicted environmental concentration" (PEC) and "predicted no-effect concentration" (PNEC). The problem is that PNEC is not as easily estimated as PEC. PEC can be calculated by applying indicators of pharmaceutical consumption, population density and other parameters required to calculate the final concentration in the environment. PNEC, however, requires the knowledge on the toxicity of each pharmaceutical and type of organism (which is already difficult to estimate, as expressed before).

1.1.4 Challenges in the detection of emerging pollutants

The problem underlying the detection of these emerging pollutants that they occur at significantly low concentrations but, as previously explained, can have a significant impact on the environment, particularly on the aquatic organisms, despite the low concentrations they are found in.

Thus, the analytical methods require an optimization for the detection and quantification of pollutants in low concentration ranges, sometimes in the ng/L range. This can be done by the use of highly sensitive equipment and methods, or by the pre-concentration of a sample, or a combination of both. The separation and the detection of an analyte just by itself are distinct and each have their own obstacles. *E.g.*, gas chromatography is very efficient for the separation of volatile substances and cannot be used for the separation of analytes dissolved in aqueous solutions, whereas liquid chromatography can be used for the separation of those.

The matrices in which the analyte is present also pose some difficulties, as its nature may interfere with the equipment's detection efficiency. For instance, depending on the type of mobile phase used in liquid chromatography, the injection of a sample dissolved in an unknown matrix may cause the precipitation of salts inside the equipment, potentially causing serious damages (Kromidas 2000).

Additionally, and specifically addressing the pharmaceutical drugs, metabolites and degradation products must also be considered when studying the presence of these substances in the environment, as the metabolites may be just as toxic as the parent-compound.

1.2 The antidepressant Sertraline

Sertraline is an antidepressant pharmaceutical that belongs to the category of the SSRIs of antidepressants, and is typically used in the treatment of major depressive disorders, obsessive-compulsive disorders, anxiety disorders, among others.

From a pharmacokinetic point of view, sertraline is extensively metabolized in the liver, achieving its peak concentration in plasma in 4.5 to 8.4 hours (Ciraulo *et al.* 2011). Its main active metabolite is known as norsertraline (or N-desmethylsertraline) and, whereas sertraline is one of the most effective SSRI as a serotonin transporter, its metabolite is considered inactive as an antidepressant (Fabre *et al.* 1995) and about 20 times less potent than its parent-compound. Sertraline's half-life in a healthy human body is estimated to be of 26 h (62 to 104 h for its main metabolite) (Ciraulo *et al.* 2011).

After its ingestion, sertraline is metabolized by enzymes in the liver, resulting in its main metabolite, N-desmethylsertraline which in turn is oxidatively deaminated into a desmethylsertraline ketone. Afterwards, it is hydroxylated into an alpha-hydroxyketone and an alcohol (WHO 1997). Throughout these reactions, only a small percentage of the parent-compound (<0.2%) is excreted in the urine.

Sertraline's chemical formula is $C_{17}H_{17}Cl_2N$ (Figure 1.4a) and has a molecular weight of 306.23 g/mol. Norsertraline's chemical formula is $C_{16}H_{15}Cl_2N$ (Figure 1.4b) and has a molecular weight of 292.203 g/mol (Table 1.1). It is mainly produced in the form of a salt, Sertraline Hydrochloride (the HCl is added in order to favour its dissolution in aqueous solutions).

Table 1.1 – Chemical formula, CAS number and molecular weight of sertraline and its main metabolite, norsertraline.

Substance	Chemical formula	CAS number	Molecular weight [g/mol]
Sertraline	$C_{17}H_{17}Cl_2N$	79617-96-2	306.23
Norsertraline (N-desmethylsertraline)	$C_{16}H_{15}Cl_2N$	87857-41-8	292.20

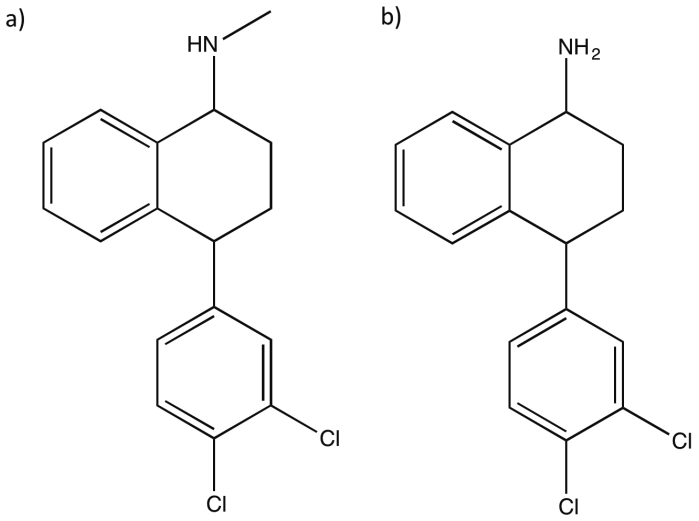

Figure 1.4 – Chemical structure of a) sertraline and b) norsertraline.

Sertraline's capacity for adsorption onto soil and sediments can be evaluated by two parameters: *log* K_{OC}, organic carbon adsorption coefficient, and *log* K_{OW}, octanol-water partition coefficient.

Log K_{OC} indicates the amount of a substance that is adsorbed onto a soil *per* mass of organic carbon present in the soil, *i.e.*, whether the substance is likely to be found in water or on the organic carbon portion of soil and sediments (Johnson *et al.* 2005). Substances with values of *log* K_{OC} estimated to be higher than 4.5 are considered to be likely to be removed through sorption from the aqueous phase in the environment (U.S. EPA 2000). Sertraline was estimated to have a *log* K_{OC} value of 5.7 by Johnson *et al.* (2005) recurring to the model PCKOCWIN. Kwon & Armbrust (2008) calculated the same value for two sediments and three soils and obtained the *log* K_{OC} value of 4.2 ± 0.4.

Log K_{OW} indicates the concentration of a substance in an octanol phase (a non-polar solvent) per concentration of substance in an aqueous phase (a polar solvent) (U.S. EPA 2000). This coefficient can be used as a "relative indicator of the tendency of an organic compound to adsorb to soil", and the higher the value, the more non-polar the substance is (U.S. Geological Survey 2015b). Sertraline was estimated to have *log* K_{OW} value of 5.3 (Christensen *et al.* 2007), and another study carried out by Kwon & Armbrust (2008) estimated the *log* K_{OW} value of 1.4 ± 0.1 (resorting to a method developed by U.S. EPA (1982)), explaining that the low value obtained was likely due to the high solubility of sertraline. The differences between the values measured by the two studies may have been because of its estimation for different substances, sertraline and sertraline hydrochlorated (more soluble).

Overall, the values presented for these two coefficients show that sertraline has a high tendency to adsorb to organic carbon present in soil and sediments. This is an important factor in WWTP treatments where it is likely to adsorb to the sludge instead of remaining in the aqueous phase, due to its high *log* K_{OW} and *log* K_{OC}. These properties were verified in a study (Malmborg & Magnér 2015) that aimed to evaluate the effect of different treatments, typically applied in WWTPs or WTPs (water treatment plants), on sertraline (among other compounds), and concluded that sertraline partitioned strongly to the solid phase throughout the various treatments.

From an environmental point of view, sertraline has been rated as having a "moderate" risk (based on the ratio between predicted environmental concentration and the predicted concentration that causes no effects, PEC/PNEC) by the Stockholm County Council (2014). The same booklet also classified the substance as a level 3 on Persistence (ability to resist degradation in the aquatic environment), level 0 on Bioaccumulation (accumulation in adipose tissue of aquatic organisms) and level 3 on Toxicity (potential to poison aquatic organisms) – levels which rank from 0 to 3.

Considering that sertraline is extensively metabolized in the human body, when studying the main sources of this particular substance into the environment, its ingestion and excretion is most likely not the main pathway for sertraline, but might be an important source of its metabolite, norsertraline, in the environment. The scientific literature reported concentrations in environmental samples within the range of 0 to 100 ng/L, only being exceed on two occasions (110 and 120 ng/L) in wastewaters from health facilities (Table 1.2).

Table 1.2 – Reported concentrations of sertraline and norsertraline detected in environmental samples.

Substance	Concentration ± standard deviation [ng/L] *[1]	Type of sample	Date	Country	Ref.*[2]
Sertraline	21	Wastewater effluents from multiple WWTPs	Jan. to Apr.'11	USA	[1]
Sertraline	34 ± 2 / 16 ± 1	Wastewater influent / effluent	Aug. '07	USA	[2]
Sertraline	n.d.	River (upstream from WWTP)	Aug. '07	USA	[2]
Sertraline	17 ± 1 / 6 ± 1 / n.d.	River (up to 100 m downstream from WWTP)	Aug. '07	USA	[2]
Sertraline	36 (5) / 49 (9) / 33 (8)	River (at 5 / 643 / 1762 m downstream from WWTP)	Aug. to Sept. '05	USA	[3]
Sertraline	100.4 / n.d.	Wastewater influent / effluent	May to Jun. '13	Portugal	[4]
Sertraline	6.0 ± 0.4 / 5.1 ± 0.3	Wastewater influent / effluent	Jun. '07	Canada	[5]
Sertraline	6.1 ± 0.3 / 5.8 ± 0.8	Wastewater influent / effluent	Sept. '07	Canada	[5]
Sertraline	0.84 ± 0.09	River (0.5 km downstream from WWTP)	Jul. '07	Canada	[5]
Sertraline	2.4 ± 0.1	River (0.5 km downstream from WWTP)	Sept. '07	Canada	[5]
Sertraline	11 ± 2	Wastewater effluent (WWTP 1)	Feb. '07	Norway	[6]
Sertraline	8.4 ± 0.4 / 6.1 ± 0.7	Wastewater influent / effluent (WWTP 1)	Apr. '07	Norway	[6]
Sertraline	9.4 ± 0.1 / 8 ± 2	Wastewater influent / effluent (WWTP 1)	Jun. '07	Norway	[6]

Substance	Concentration ± standard deviation [ng/L] *[1]	Type of sample	Date	Country	Ref.*[2]
Sertraline	20 ± 2 / 7.9 ± 0.7	Wastewater influent / effluent (WWTP 2)	Mar. '07	Norway	[6]
Sertraline	12 ± 1 / 8.1 ± 0.4	Wastewater influent / effluent (WWTP 1)	Sept. '09	Canada	[7]
Sertraline	7.6 ± 0.7 / 5.7 ± 1.1	Wastewater influent / effluent (WWTP 1)	Apr. '10	Canada	[7]
Sertraline	26 ± 2 / 14 ± 3	Wastewater influent / effluent (WWTP 2)	Aug. '09	Canada	[7]
Sertraline	34 ± 7 / 21 ± 6	Wastewater influent / effluent (WWTP 2)	Mar. '09	Canada	[7]
Sertraline	23 ± 4 / 16 ± 7	Wastewater influent / effluent (WWTP 3)	Jul. '09	Canada	[7]
Sertraline	16 ± 8 / 8.1 ± 3.9	Wastewater influent / effluent (WWTP 4)	Aug. '09	Canada	[7]
Sertraline	78 ± 6 / n.d. / 110 ± 6 / 120 ± 20	Wastewater from hospital / nursing home / assisted living / independent living	Apr. '08	USA	[8]
Sertraline	11 / n.d.	Raw / Finished water from drinking water treatment plant	Oct. '08 to Jan. '09	Spain	[9]
Norsertraline	9.9	Wastewater effluents from multiple WWTPs	Jan. to Apr.'11	USA	[1]
Norsertraline	21 ± 3 / <LOQ	Wastewater influent / effluent	Aug. '07	USA	[2]
Norsertraline	n.d.	River (upstream from WWTP)	Aug. '07	USA	[2]
Norsertraline	5 ± 1 / n.d.	River (up to 100 m downstream from WWTP)	Aug. '07	USA	[2]
Norsertraline	5 (3) / 7 (3) / 3 (1)	River (at 5 / 643 / 1762 m downstream from WWTP)	Aug. to Sept. '05	USA	[3]
Norsertraline	5.0 ± 0.8 / 3.6 ± 0.3	Wastewater influent / effluent	Jun. '07	Canada	[5]
Norsertraline	4.2 ± 0.6 / 4.7 ± 0.5	Wastewater influent / effluent	Sept. '07	Canada	[5]
Norsertraline	2.3 ± 0.1	River (0.5 km downstream from WWTP)	Jul. '07	Canada	[5]
Norsertraline	4.5 ± 0.4	River (0.5 km downstream from WWTP)	Sept. '07	Canada	[5]
Norsertraline	<LOQ / <LOQ	Wastewater influent / effluent (WWTP 1)	Apr. '07	Norway	[6]
Norsertraline	<LOQ / <LOQ	Wastewater influent / effluent (WWTP 1)	Jun. '07	Norway	[6]
Norsertraline	31 ± 5 / 6.2 ± 0.8	Wastewater influent / effluent (WWTP 2)	Mar. '07	Norway	[6]
Norsertraline	19 ± 2 / 14 ± 2	Wastewater influent / effluent (WWTP 1)	Sept. '09	Canada	[7]
Norsertraline	15 ± 5 / 12 ± 4	Wastewater influent / effluent (WWTP 1)	Apr. '10	Canada	[7]
Norsertraline	19 ± 5 / 13 ± 2	Wastewater influent / effluent (WWTP 2)	Aug. '09	Canada	[7]
Norsertraline	19 ± 2 / 15 ± 2	Wastewater influent / effluent (WWTP 2)	Mar. '09	Canada	[7]
Norsertraline	19 ± 8 / 16 ± 7	Wastewater influent / effluent (WWTP 3)	Jul. '09	Canada	[7]
Norsertraline	30 ± 21 / 24 ± 18	Wastewater influent / effluent (WWTP 4)	Aug. '09	Canada	[7]
Norsertraline	42 ± 1 / n.d. / 86 ± 11	Wastewater from hospital / nursing home / assisted living	Apr. '08	USA	[8]

*[1] Values in parenthesis correspond to the calculated 95% confidence interval; n.d.: not detected; <LOQ: below limit of quantification.

*[2] [1] (Kostich *et al.* 2014); [2] (Metcalfe *et al.* 2010); [3] (Schultz & Furlong 2008); [4] (Silva *et al.* 2014); [5] (Lajeunesse *et al.* 2008); [6] (Vasskog *et al.* 2008); [7] (Lajeunesse *et al.* 2012); [8] (Nagarnaik *et al.* 2011); [9] (Huerta-Fontela *et al.* 2011)

1.3 Elimination of antidepressants in WWTPs

The main elimination processes in WWTPs are through adsorption onto sludge and/or biodegradation. According to the properties of each SSRI, one or the other might be more suitable for their elimination but sometimes other methods need to be employed - tertiary processes, such as ozonation, UV treatments, ultrafiltrations, reverse osmosis, among others (Sui *et al.* 2010; Behera *et al.* 2011; Zupanc *et al.* 2013).

Pharmaceutical substances more prone to adsorbing to particulate matter (the more hydrophobic) will likely be retained in the sludge, which is in some cases applied as soil amendment, so this must be taken into consideration when "removing" a drug from the aquatic systems.

Biodegradation processes are more important for pharmaceuticals which occur mainly on the aqueous phase. Depending on the concentrations of the pharmaceutical and the performance of the process, microorganisms present in typical aerated activated sludge biological processes may degrade the substance efficiently.

When none of the processes (adsorption onto sludge and biodegradation) are sufficient for the elimination of the drug, processes like solar irradiation (such as the one further explored in this study) may render high efficiencies of treatment. The photodegradation process is particularly important on surface waters since, aside from biodegradation, solar irradiation is the natural alternative solution for the substance to be adequately removed.

There are only a few studies on removal efficiencies of sertraline by WWTP typically applied treatments (see Table 1.3). From the published and peer-reviewed studies, it is shown that sertraline has an average removal rate lower than 50%. The solid and hydraulic retention times (SRT and HRT, respectively) are shown to influence the removal efficiencies of sertraline (Metcalfe *et al.* 2010; Lajeunesse *et al.* 2012); however, whether the increase of SRT (Solids Retention Time) or HRT (Hydraulic Retention Time) does cause an increase in removal efficiencies is inconclusive.

Table 1.3 – Removal efficiencies of sertraline in water treatments applied in WWTPs.

WWTP description *[1]	Removal efficiency (%) *[1]	Ref. *[2]
Conventional activated sludge, tertiary treatment and UV-disinfection Average flow of 0.34 m^3/s and HRT of 11.9 h	29% Aeration with high SRT (10.4 d)	[1]
	53% Aeration with low SRT (8.1 d)	
Activated sludge with extended aeration, tertiary treatment with UV Flow of 134 m^3/day	100%	[2]

WWTP description *[1]	Removal efficiency (%) *[1]	Ref. *[2]
Physicochemical treatment by flocculation and sedimentation of suspended matter Average flow of 19.8 m^3/s	15% June 2007 (Summer period)	[3]
	5% September 2007 (Autumn period)	
Secondary treatment with biological nutrient removal Average flow of 4.63 m^3/s	34% SRT of 7.5 d and HRT of 23 h (September 2009)	[4]
	25% SRT of 18 d and HRT of 26.7 h (April 2010)	
Secondary treatment with biological aerated filter Average flow of 0.69 m^3/s	46% HRT of 5 h (August 2009)	[4]
	37% HRT of 6.8 h (March 2009)	
Secondary treatment with trickling filter and solid contact Average flow of 5.21 m^3/s and HRT of 10.8 h	33%	[4]
Secondary treatment with activated sludge Average flow of 2.62 m^3/s and HRT of 12.6 h	48%	[4]
Primary treatment chemically enhanced with aluminium and ferric chloride Average flow of 0.32 m^3/s and HRT of 2.4 h	26%	[4]
Anaerobic treatment (simulation at a laboratory scale using raw sludge) Average flow of 400 mL/day	38% (after 24 days of operation)	[5]

*[1] SRT: Solids Retention Time; HRT: Hydraulic Retention Time

*[2] [1] (Metcalfe *et al.* 2010); [2] (Silva *et al.* 2014); [3] (Lajeunesse *et al.* 2008); [4] (Lajeunesse *et al.* 2012); [5] (Bergersen *et al.* 2012)

1.4 Photodegradation of pharmaceuticals

In aquatic environments, the fate of pharmaceuticals is determined by biological processes, abiotic processes, namely photolysis and hydrolysis, and sorption to particles or sludge (Styrishave *et al.* 2011). *Ergo* studies on the photodegradation of pharmaceuticals provide essential information for the estimation of its persistence on the environment.

In natural surface waters, pharmaceutical compounds can undergo direct and/or indirect photodegradation processes. Direct photodegradation consists on the absorption of radiation by the compound, leading to its degradation and formation of photoproducts. Since sertraline does not absorb significantly above the 290 nm, it is unlikely that it can be photodegraded solely under the direct influence of solar light, since this falls below the spectrum of 290-800 nm. Thus, its degradation depends strongly on the indirect photodegradation, which consists on the absorption of the radiation by other molecules present in the matrix, leading to the formation of reactive species that will act on the pharmaceutical compound, degrading it.

Thus far, there are only a few published and peer-reviewed studies on the photodegradation rate under solar irradiation of SSRIs, namely citalopram (CIT), fluoxetine (FLX), fluvoxamine (FVX),

paroxetine (PXT) and sertraline (SER) (see Table 1.4). These studies were all performed under different irradiation conditions; some resort to natural sunlight which has a different intensity depending on the location in the globe where the study was conducted as well as the season (summer is characterized by longer periods of sunlight and more intense irradiance levels, whereas winter has shorter periods of sunlight and the irradiation is not as intense), and others resort to simulated sunlight (either UV lamps, Xenon lamps or LED lamps). Regarding sertraline, the information provided by each study (Styrishave *et al.* 2011; Jakimska *et al.* 2014) does not allow a direct comparison of results, but both reached degradation times superior to 4 days, either in natural superficial water bodies or wastewater. Considering the continuous discharge of pharmaceutical substances to the environment, this is a long period for it to degrade naturally, which results in its accumulation in the environment or eventual sorption onto the particulate matter or river soils.

Fluvoxamine and paroxetine seem to be the two SSRIs with the fastest degradation due to solar irradiation, both with half-life times equivalent to less than a day. On the other hand, citalopram and fluoxetine both seem to be very hard to degrade recurring to photodegradation processes.

Table 1.4 – Photodegradation studies of SSRIs in different matrices and irradiation conditions, initial concentration (C_0), observed photodegradation rate (k_{obs}) and half-life times ($t_{1/2}$).

Subst. *[1]	Matrix	$k_{obs} \pm \sigma$ *[2]	$t_{1/2} \pm \sigma$ *[2]	C_0 *[2]	Irradiation conditions	Ref. *[4]
CIT	Synthetic humic water	0.028 d^{-1}	24 d	5 mg/L	Simulated sunlight UV lamps (> 290nm)	[1]
CIT	Lake water 1 (filtered by 0.2 μm)	0.051 d^{-1}	14 d	5 mg/L	Simulated sunlight UV lamps (> 290nm)	[1]
CIT	Lake water 2 (filtered by 0.2 μm)	0.016 d^{-1}	43 d	5 mg/L	Simulated sunlight UV lamps (> 290nm)	[1]
CIT	Sewage sludge (autoclaved and filtered by 0.2 μm)	n/a	53-462 d	25 mg/L	Simulated sunlight 2 x 15 W 5, 20 and 40 °C variation	[2]
FLX	Ultrapure water (pH 5.5)	0.0126 ± 0.001 h^{-1}	55.2 ± 3.6 h	10 μM	Simulated sunlight Xe-lamp at 765 W/m^2 (> 290nm)	[3]
FLX	Ultrapure water	n/a	7 ± 1 d	10 μM	Natural sunlight (Toronto, Canada)	[3]
FLX	Synthetic field water 1 (8 μM NO^{3-}, 0.7 mg/L DOC, 0.8 mM HCO^{3-}, pH 8)	n/a	22 ± 0.9 h	10 μM	Simulated sunlight Xe-lamp at 765 W/m^2 (> 290nm)	[3]
FLX	Synthetic field water 2 (81 μM NO^{3-}, 7 mg/L DOC, 5 mM HCO^{3-}, pH 10)	n/a	5.5 ± 0.3 h	10 μM	Simulated sunlight Xe-lamp at 765 W/m^2 (> 290nm)	[3]
FLX	Synthetic humic water	0.034 ± 0.001 d^{-1}	21 ± 1 d	5 mg/L	Simulated sunlight UV lamps (> 290nm)	[4]

Subst. *[1]	Matrix	$k_{obs} \pm \sigma$ *[2]	$t_{1/2} \pm \sigma$ *[2]	C_0 *[2]	Irradiation conditions	Ref. *[4]
FLX	Lake water 1 (filtered by 0.2 μm)	0.006 ± 0.000 d^{-1}	112 ± 5 d	5 mg/L	Simulated sunlight UV lamps (> 290nm)	[4]
FLX	Lake water 2 (filtered by 0.2 μm)	0.005 ± 0.000 d^{-1}	133 ± 6 d	5 mg/L	Simulated sunlight UV lamps (> 290 nm)	[4]
FLX	Sewage sludge (autoclaved and filtered by 0.2 μm)	n/a	44-99 d	25 mg/L	Simulated sunlight 2 x 15 W 5, 20 and 40 °C variation	[2]
FVX	Synthetic humic water	1.34 d^{-1}	0.52 d	5 mg/L	Simulated sunlight Xe-lamp at 765 W/m^2 (>290 nm)	[5]
FVX	Lake water 1 (filtered by 0.2 μm)	1.28 d^{-1}	0.54 d	5 mg/L	Simulated sunlight Xe-lamp at 765 W/m^2 (>290 nm)	[5]
FVX	Lake water 2 (filtered by 0.2 μm)	1.15 d^{-1}	0.60 d	5 mg/L	Simulated sunlight Xe-lamp at 765 W/m^2 (>290 nm)	[5]
PXT	Synthetic humic water	0.0466 ± 0.0010 h^{-1}	14.92 ± 0.32 h	5 mg/L	Simulated sunlight UV lamps (>290 nm)	[6]
PXT	Lake water 1 (filtered by 0.2 μm)	0.0437 ± 0.0010 h^{-1}	15.87 ± 0.36 h	5 mg/L	Simulated sunlight UV lamps (>290 nm)	[6]
PXT	Lake water 2 (filtered by 0.2 μm)	0.0411 ± 0.0004 d^{-1}	16.84 ± 0.17 h	5 mg/L	Simulated sunlight UV lamps (>290 nm)	[6]
SER	Sewage sludge (autoclaved and filtered by 0.2 μm)	n/a	4-11 d	25 mg/L	Simulated sunlight 2 x 15 W 5, 20 and 40 °C variation	[2]
SER	WWTP influent (pH 6.9)	0.100 d^{-1}	6.9 d	1 mg/L	Natural sunlight (Poland)	[7]
SER	WWTP effluent (pH 7.5)	0.140 d^{-1}	4.9 d	1 mg/L	Natural sunlight (Poland)	[7]
SER	River water (pH 7.9)	0.100 d^{-1}	6.9 d	1 mg/L	Natural sunlight (Poland)	[7]
SER	Untreated water (pH 7.4)	0.067 d^{-1}	10.3 d	1 mg/L	Natural sunlight (Poland)	[7]
SER	Treated water (pH 8.2)	0.041 d^{-1}	14 + 16.8 d *[3]	1 mg/L	Natural sunlight (Poland)	[7]
SER	Ultrapure water (pH 3)	0.005 d^{-1}	120 + 127.5 d *[3]	1 mg/L	Natural sunlight (Poland)	[7]
SER	Ultrapure water (pH 10)	0.071 d^{-1}	9.7 d	1 mg/L	Natural sunlight (Poland)	[7]
SER	River water (pH 7.9)	0.009 min^{-1}	76.5 min	1 mg/L	Simulated sunlight Xe-lamp at 1000 W (250-1000 nm)	[7]

*[1] Substances CIT: Citalopram; FLX: Fluoxetine; FVX: Fluvoxamine; PXT: Paroxetine; SER: Sertraline

*[2] k_{obs}: observed photodegradation rate; $t_{1/2}$: half-life time; C_0: initial concentration of the irradiation procedures; σ: standard deviation; n/a: not available.

*[3] $t_{1/2}$ correspond to delay time + half-life time (autocatalytic reaction)

*[4] [1] (Kwon & Armbrust 2005a); [2] (Styrishave et al. 2011); [3] (Lam et al. 2005); [4] (Kwon & Armbrust 2006); [5] (Kwon & Armbrust 2005b); [6] (Kwon & Armbrust 2004); [7] (Jakimska et al. 2014)

1.4.1 Direct photodegradation

Schwarzenbach *et al.* (2003) defines direct photodegradation as a process "in which a given organic pollutant absorbs light and, as a consequence of that light absorption, undergoes transformation".

It is important to note that this process is dependent on the intensity and frequency of the light exposure, *i.e.* the photolysis will not occur in water surfaces that are shadowed by trees, will not be as efficient in turbid waters and is also distinct at different depths of water. However, indirect photodegradation might happen in those cases (Kümmerer 2010).

The absorption of radiation energy by the substance may lead to higher degradation rates when the substance itself has a high absorptivity at the visible wavelength range (which can be verified through the analysis of the adsorption spectrum performed in the wavelength range of 290-800 nm). However, the quantum yield of the substance determines how many of the molecules of the substance actually undergo a chemical transformation per total number of absorbed photons (Schwarzenbach *et al.* 2003). Therefore, a substance of low visible light absorptivity may be photodegraded if it has a high quantum yield, and vice versa, a substance of low quantum yield and high visible light absorptivity may not photodegrade as easily.

1.4.2 Indirect photodegradation

Indirect photodegradation relies on the reaction of the pollutants with photoreactants (very reactive, short-lived species formed in the presence of light, such as hydroxyl radicals, singlet oxygen, ozone, peroxy radicals (Schwarzenbach *et al.* 2003)), as well as energy transfer from excited species (otherwise known as photosensitizers). Photosensitization is defined as the transfer of energy from an excited species to a nearby molecule (Schwarzenbach *et al.* 2003) and is one of the main pathways of indirect photodegradation.

Figure 1.5 summarizes the main processes by which some photosensitizers, dissolved organic matter (DOM) in particular, promote the photodegradation of organic compounds.

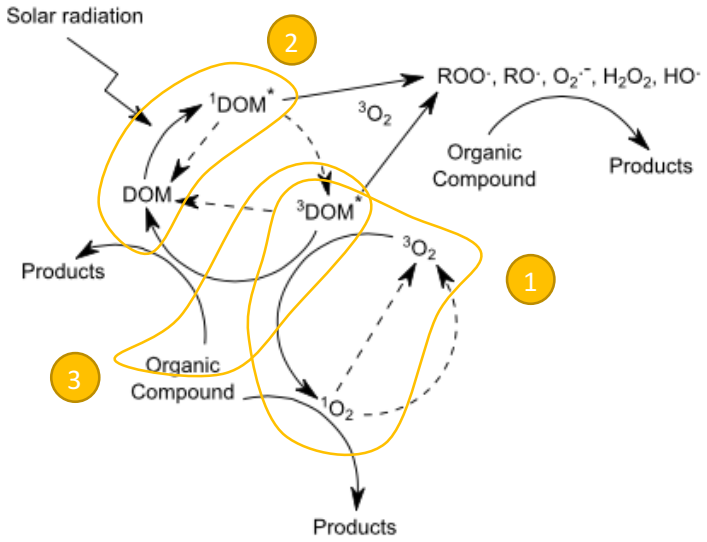

Figure 1.5 – Main processes of indirect photodegradation of organic compounds. Adapted from Calisto (2011).

One of the phenomena here portrayed (1) is the transfer of the absorbed light energy by a chromophore (present in the dissolved organic matter) to the oxygen molecule ($^3O_2$), promoting it to its first excited state ($^1O_2$). The singlet oxygen then reacts with the organic compound, leading to its degradation.

Simultaneously, by effect of the solar radiation (2), DOM can be promoted to their excited singlet state (^{1}DOM*) through the direct absorption of light energy (Calisto 2011). Then, naturally, this singlet state is converted to a more stable state, ^{3}DOM* (triplet state), through a radiationless process, as described by Eq. 1.1.

$$DOM \overset{h\upsilon}{\rightarrow} {}^1DOM^* \rightarrow {}^3DOM^* \qquad \text{Eq. 1.1}$$

Meanwhile ^{3}DOM* may also react directly with the organic compound (3) leading to the degradation of the organic compound. Nonetheless, because ^{3}DOM* is more stable than the other states of DOM, it is more likely to react with more easily oxidizable compounds (Schwarzenbach *et al.* 2003).

1.4.3 Importance of photosensitizing agents

1.4.3.1 Humic substances

According to Stevenson (1994), cited by Messias (2004), humic substances (HS) are an heterogeneous mixture of polydispersed molecules of high molecular mass, with several distinct functional groups, and are responsible for numerous natural processes, such as improving soil fertility, influencing the colour of water or soil, or degrading natural organic chemicals and other pollutants (IHSS 2007).

These substances are among the major components of natural organic matter (NOM), which, by itself is difficult to replicate in laboratory. For this reason, HS are very useful in experiments where, for instance, the influence of natural organic matter is yet to be discovered. A natural water sample may contain several other constituents which may influence the experiment at hand, and in these cases, it is helpful to use HS as a fair analytical representation of NOM.

HS can be divided into different categories, depending on the method of extraction as well as their main properties: humic acids (HA), fulvic acids (FA), hydrophilic fractions and humins:

- Humic acids are the fraction that is soluble only at high pH levels and is obtained as a precipitate after the process of acidification and centrifugation.
- Fulvic acids are the fraction of lowest molecular weight and are soluble at all pH levels, corresponding to the part that remains after the isolation of humic acids.
- Hydrophilic fractions are isolated from samples after the extraction of the most hydrophobic fractions (HA and FA).
- Humins are not soluble at any pH level and, accordingly, cannot be extracted with neither a strong base nor a strong acid.

The humic substances can be isolated through a process that includes XAD-8 resins and series of acidifications and alkalinizations in order to separate the various fractions. The process by which the substances used throughout this study were isolated is presented in Esteves (1995). After the isolation of HA, an additional step was taken for the isolation of hydrophilic organic acids, by applying XAD-4 styrene in series after the XAD-8 resins (Esteves *et al.* 2009). These substances are referred to as XAD-4 fraction.

The absorption of solar radiation by humic substances (and other organic matter) leads to transformations at a molecular level as well as the formation of several reactive species (Schwarzenbach *et al.* 2003; Chen *et al.* 2013). These reactive species come under the form of

singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet-}$), hydroperoxyl radical ($HO_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydrated electrons (e^-_{aq}) and reactive HS triplet states (^{3}HS*). The formation of some of these reactive species, during the irradiation process of humic acids follows the mechanisms presented below, proposed by Haag & Hoigne (1986), Cooper *et al.* (1988) and Vaughan & Blough (1998) (cited by Chen *et al.* (2013)):

$$HA + h\upsilon \rightarrow {}^1HA^* \rightarrow {}^3HA^* \quad \text{Eq. 1.2}$$

$${}^3HA^* + O_2 \rightarrow HA + {}^1O_2 \quad \text{Eq. 1.3}$$

$${}^3HA^* + O_2 \rightarrow HA^+ + O_2^{\cdot-} \quad \text{Eq. 1.4}$$

$$2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{Eq. 1.5}$$

$$H_2O_2 + h\upsilon \rightarrow 2\,HO\cdot \quad \text{Eq. 1.6}$$

$$HA/\ {}^1HA^*/\ {}^3HA^*/HA^+ + HO\cdot \rightarrow Oxidized\ HA \quad \text{Eq. 1.7}$$

High concentrations of organic matter may lead to an effect of light screening, by absorbing the photons emitted by the light source, preventing the photons from reaching the contaminant. Additionally to the inhibition of photodegradation of the contaminant, organic matter may also quench the reactive species that were formed as a result of its oxidation (Eq. 1.7). Thus, the intended photosensitizing effect may be suppressed depending on the type and concentration of organic matter.

Chen *et al.* (2013) concluded that the photoactivated species of HA (^{1}HA*, ^{3}HA*, HA^+) are quenchers of free radicals (more effectively than the parent HA), which means that the photoproducts of the humic substances had an impact in the indirect photodegradation of the studied pollutant (the hormone estriol, E3, for this particular case study).

1.4.3.2 Ferric substances

The use of ferric substances for the oxidation of organic compounds under irradiation is commonly associated with the photo-fenton chemical reaction. This process consists of a photochemical reduction of Fe(III) to Fe(II) in aqueous solution through the absorption of photons in the UV-visible wavelength range (Eq. 1.8). This chemical reaction also leads to the formation of the photoreactants $HO^{\bullet}$ and H^+. Subsequently, these photoreactants proceed to the oxidation of organic compounds present in the solution.

$$Fe(III) + H_2O + h\upsilon \rightarrow Fe(II) + HO\cdot + H^+ \quad \text{Eq. 1.8}$$

This process is pH dependant, as the speciation of Fe(III) is a function of pH: at pH<2 the dominant species are Fe^{3+} (absorbs weakly at wavelengths > 300nm), at pH 3 the dominant species are $FeOH^{2+}$ (absorbs mostly in the UV wavelength range) and at pH>3 the dominant species are $Fe(OH)_2^+$ (which are very prone to precipitation) (Machulek *et al.* 2012).

The photosensitizing effect of ferric complexes has been verified in previous studies (Chen *et al.* 2013; Schwarzenbach *et al.* 2003) and it was noted that the ferric substances in nature are more commonly found bound to an organic complex. Chen *et al.* (2013) investigated the photochemical behaviour of a hormone (Estriol, E3) in the presence of several photosensitizers, among which were the Fe(III)-carboxylate species. This complex was chosen due to its extensive presence in natural waters.

The presence of the organic complex allows the formation of H_2O_2 (Eq. 1.9 to Eq. 1.11), which reacts with Fe(II) oxidising it back to Fe(III) (Eq. 1.12), and the reactions carry on continuously due to the regeneration of the ferric substances and the production of H_2O_2 which strongly influences the production of photoreactants like HO• and OH^- (Schwarzenbach *et al.* 2003; Zuo & Hoigné 1992; Chen *et al.* 2013; Machulek *et al.* 2012).

$$Fe(III) - organiccomplex + h\upsilon \rightarrow Fe(II) + Org\cdot \quad \text{Eq. 1.9}$$

$$Org\cdot + O_2 \rightarrow Oxidized\ Org + O_2^{\cdot -} \quad \text{Eq. 1.10}$$

$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{Eq. 1.11}$$

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + HO\cdot + OH^- \quad \text{Eq. 1.12}$$

The effect of this photosensitizer is greatly affected by the solution's pH, as mentioned previously, and it has been verified that the weakly acidic pH levels facilitate the photodegradation of the hormone E3 in Fe(III)-oxalate solutions (Chen *et al.* 2013). That study used Fe(III)-oxalate as representative of the Fe(III)-carboxylate complexes, having prepared a Fe(III)-oxalate solution with 10 μM Fe(III) and 150 μM oxalate.

Sertraline is an organic compound, so the reactions described above may occur in solutions composed of Fe(III) and sertraline, without the need for the addition of an organic complex like oxalate to catalyse the process.

1.4.3.3 Nitrates and nitrites

Nitrate absorbs in the range λ < 350 nm and reaches its maximum at 302 nm, which means that it is very reactive under sunlight (Chen *et al.* 2013). Nitrite is photoactive and photochemically unstable, especially with organic matter and, comparatively to nitrate, absorbs in a larger fraction of the solar spectrum, which means that it is an important source of HO• in salt water, but it may also lead to the formation of harmful compounds (Calza *et al.* 2012).

One of the major sources of HO• radicals is thought to be the photolysis of NO_3^- and NO_2^- (Schwarzenbach *et al.* 2003):

$$NO_3^- + h\upsilon \rightarrow NO_3^{-*} \rightarrow NO_2 + O^{\cdot -} \xrightarrow{H_2O} HO\cdot + OH^- \quad \text{Eq. 1.13}$$

$$NO_2^- + h\upsilon \rightarrow NO_2^{-*} \rightarrow NO + O^{\cdot -} \xrightarrow{H_2O} HO\cdot + OH^- \quad \text{Eq. 1.14}$$

As well as with the ferric species, the photosensitizing effect of NO_3^- also appears to be pH dependent. In previous studies, the photodegradation of E3 increased with increasing pH level in the range of 6.0 to 10.0 in the presence of NO_3^- (Chen *et al.* 2013). It was concluded that "the HO• produced was exclusively responsible for the indirect degradation of E3" (Chen *et al.* 2013).

On another study (focused on the photodegradation of phenol), it was concluded that the presence of nitrate and nitrite ions lead to the formation of more photoproducts that, incidentally, were more toxic than the parent compound (Calza *et al.* 2012).

1.5 Analytical methods of detection/quantification

To this date, several methods have been developed for the detection and quantification of the antidepressant sertraline in different types of samples (see Table 1.5). The chromatography analytical methods rely mostly on HPLC with C18 columns, differing mostly on the mobile phase and the type of detector used (which has been either a UV detector or a mass spectrometer). Solid-phase extraction (SPE) has also been used to allow for a pre-concentration and cleaning of the sample, allowing a detection at a lower concentration range.

Table 1.5 – Analytical methods based on high performance liquid chromatography with UV detection of sertraline described in the literature.

Column specifications *[1]	Operating conditions *[1]	Detector conditions *[1]	Method parameters *[1]	Analysed substances*[2]	Ref.*[3]
C_{18} column (25 cm x 4.6 mm *i.d.* x 5 µm) C_{18} guard-column (4.5 cm x 4.6 mm *i.d.* x 5 µm)	*M.P.*: acetonitrile and sodium phosphate buffer (0.05 M, pH 3.80) (50:50, v/v) f_R: 1 mL/min *T*: 50°C V_{inj}: 50 µL	Diode array detector λ: 200.4 nm	t_r: 13.89 min LOD: 40 ng LOQ: n/a	FVX, FLX, **SER**, PXT, CIT, MIL, VNF	[1]
C_{18} column (250 mm x 4.6 mm *i.d.* x 5 µm)	*M.P.*: acetonitrile (A) and potassium monophosphate buffer (40 mM, pH 3) (B) (gradient: 15% A for 6 min, 40% A for 10 min, 75% A for 5 min) f_R: 1.2 mL/min *T*: n/a V_{inj}: 100 µL	Diode array detector λ: 214 nm	t_r: 22.5 min LOD: 1.9 µg/L LOQ: 7.5 µg/L	ACT, AVA, CAF, CBZ, LVX, **SER**, SMX, TMP	[2]
C_{18} column (250 mm x 4.6 mm *i.d.* x 5 µm)	*M.P.*: methanol and water (75:25, v/v) f_R: n/a *T*: n/a V_{inj}: 100 µL	UV detector λ: 273 nm	t_r: 7.05 min LOD: 28 ng/mL LOQ: 85.5 ng/mL	**SER**	[3]
C_{18} column (150 mm x 4.6 mm *i.d.* x 5 µm)	*M.P.*: methanol and phosphate buffer (10 mM, pH 2.8) (37:63, v/v) f_R: 1 mL/min *T*: 50°C V_{inj}: 7 µL	UV detector λ: 220 nm	t_r: n/a LOD: n/a LOQ: n/a	**SER**, impurities	[4]
Silica column (150 mm x 4.6 mm *i.d.* x 5 µm)	*M.P.*: methanol and ammonium perchlorate (40 mM, ph 7.0) (95:5, v/v) f_R: 1.2 mL/min *T*: n/a V_{inj}: 200 µL	UV detector λ: 215 nm	t_r: n/a LOD: n/a LOQ: n/a	**SER**, DMS	[5]
C_8 column (150 mm x 4.6 mm *i.d.* x 4 µm)	*M.P.*: acetonitrile and phosphate buffer (12.3 mM, pH 3.0) (35:65, v/v) f_R: 1.2 mL/min *T*: n/a V_{inj}: 20 µL	UV detector λ: 220 nm	t_r: 12.20 min LOD: 7.5 ng/mL LOQ: 2.5 ng/mL	**SER**, DMS	[6]
C_{18} column	*M.P.*: acetonitrile and potassium phosphate buffer (0.25M, pH 2.7) (30:70, v/v) f_R: 2.0 mL/min *T*: n/a V_{inj}: n/a	UV detector λ: 235 nm	t_r: n/a LOD: n/a LOQ: n/a	**SER**, DMS	[7]
C_{18} column (250 mm x 4.6 mm *i.d.* x 5 µm)	*M.P.*: acetonitrile and phosphate buffer (0.085 M, pH 3.5) (29:71, v/v) f_R: 2 mL/min *T*: n/a V_{inj}: n/a	UV detector λ: 232 nm	t_r: 12.1 ± 0.2 min LOD: 15 nmol LOQ: n/a	**SER**, DMS	[8]
C_{18} column (250 mm x 4.6 mm *i.d.* x 5 µm)	*M.P.*: acetonitrile and phosphate buffer (pH 3.8) f_R: 1.0 mL/min *T*: n/a V_{inj}: n/a	Diode array detector λ: 220, 240 and 290 nm	t_r: n/a LOD: n/a LOQ: n/a	CIT, FLX, FVX, MCB, MIL, MIR, NFL, ODV, PXT, **SER**, TLX, VLX, VNF	[9]

Column specifications *[1]	Operating conditions *[1]	Detector conditions *[1]	Method parameters *[1]	Analysed substances*[2]	Ref.*[3]
C_8 column	*M.P.*: acetonitrile and phosphate buffer (10 mM, pH 3.8) f_R: n/a *T*: n/a V_{inj}: n/a	Diode array detector λ: 230 and 290 nm	t_r: n/a LOD: n/a LOQ: 25 ng/mL	CIT, DCT, DDCT, DMIR, FLX, FVX, MIL, MIR, NFL, ODV, PXT, **SER**, VNF	[10]
Nucleosil 100-5-Protect 1 column (250 mm x 4.6 mm *i.d.* x 5 µm)	*M.P.*: acetonitrile and potassium dihydrogenphosphate (25 mM, pH 7.0) (60:40, v/v) f_R: 1 mL/min *T*: 25°C V_{inj}: 100 µL	UV detector λ: 230 nm	t_r: 33.6 min LOD: n/a LOQ: n/a	MIR, RBX, MCB, VNF, ODV, PXT, FLX, FVX, NFL, **SER**, CIT, AMT, NTP, RMI, DMI, DOX, NDX, CLI, NCL, TRI, MIA, MPT, NMP, ASP, CLZ, NCZ, QTP, RIS, 9-OH-RIS	[11]
LiChrospher 60 RP-select B column (250 mm x 4 mm *i.d.* x 5 µm)	*M.P.*: 35% [acetonitrile: methanol (92:8, v/v)] and 65% sodium acetate buffer (0.25 M/L, pH 4.5) f_R: 1.0 mL/min *T*: n/a V_{inj}: 100 µL	UV detector λ: 230 nm	t_r: 24 min LOD: n/a LOQ: 10 ng/mL	AMT, CIT, CLI, DUL, FLX, IMI, MCB, MIR, PXT, **SER**	[12]
C_{18} column (100 mm x 2.1 mm *i.d.* x 3.5 µm)	*M.P.*: methanol and acetic acid solution (0.02 M, pH 4) (46:56, v/v) f_R: 0.25 mL/min *T*: n/a V_{inj}: 20 µL	UV detector λ: 215 nm	t_r: 20 min LOD: 0.7 µg/L LOQ: 2.3 µg/L	AMT, IMI, **SER**	[13]
C_{18} column (250 mm x 4.6 mm *i.d.* x 5 µm)	*M.P.*: hydroxypropyl-β-cyclodextrin in acetonitrile (18 nM) and aqueous phosphate buffer (170 mM, pH 3.0) (32:68, v/v) f_R: 1.0 mL/min *T*: 24°C V_{inj}: 20 µL	UV detector λ: 225 nm	t_r: 19 min LOD: 0.029 µg/mL LOQ: 0.097 µg/mL	**SER**	[14]
C_{18} column (250 mm x 4 mm *i.d.* x 5 µm)	*M.P.*: acetonitrile and phosphate buffer (0.05 mol/L, pH 3.8) (47:53, v/v) f_R: 1.0 mL/min *T*: 25°C V_{inj}: 250 µL	Diode array detector λ: 230 nm	t_r: 13.08 min LOD: 20 ng/mL LOQ: 40 ng/mL	CIT, DUL, FLX, MIR, PXT, **SER**	[15]

*[1] *i.d.*: inner diameter; n/a: not available; *M.P.*: mobile phase; f_R: flow rate; *T*: column temperature; V_{inj}: injection volume; t_r: retention time; LOD: limit of detection; LOQ: limit of quantification

*[2] ACT: Acetaminophen; AMT: amitriptyline; ASP: Amilsupride; AVA: Atorvastatin; CAF: Caffeine; CBZ: Carbamazepine; CIT: Citalopram; CLI: Clomipramine; CLZ: Clozapine; DCT: Desmethylcitalopram; DDCT: Didesmethylcitalopram; DMI: Desipramine; DMIR: Desmethylmirtazepine; DMS: N-Desmethylsertraline; DOX: Doxepin; DUL: Duloxetine; FLX: Fluoxetine; FVX: Fluvoxamine; IMI: Imipramine; LVX: Levofloxatin; MCB: Moclobemide; MIA: Mianserin; MIL: Milnacipram; MIR: Mirtazepine; MPT: Maprotiline; NCL: Norclomipramine; NCZ: Norclozapine; NDX: Nordoxepin; NFL: Norfluoxetine; NMP: Normaprotiline; NTP: Nortriptyline; ODV: O-Desmethylvenlafaxine; PXT: Paroxetine; QTP: Quetiapine; RBX: Reboxetin; RIS: Risperidone; SER: Sertraline; SMX: Sulphamethoxazole; TLX: Toloxatone; TMP: Trimethoprim; TRI: Trimipamine; VLX: Viloxazine; VNF: Venlafaxine

*[3] [1] (Tournel *et al.* 2001); [2] (Lam *et al.* 2004); [3] (Rahman *et al.* 2012); [4] (Ferrarini *et al.* 2010); [5] (Patel *et al.* 1996); [6] (Mandrioli *et al.* 2006); [7] (Wiener *et al.* 1990); [8] (Vatassery *et al.* 1997); [9] (Duverneuil *et al.* 2003); [10] (Titier *et al.* 2003); [11] (Frahnert *et al.* 2003); [12] (Malfará *et al.* 2007); [13] (Esrafili *et al.* 2007); [14] (Chen *et al.* 2004); [15] (Chen *et al.* 2003)

1.6 Objectives and Motivations

The present study is focused on the photodegradation of the antidepressant sertraline, under simulated solar radiation, and the influence of different environmentally relevant factors. In light of this, four main objectives were established throughout this study:

Firstly, the development and optimization of an analytical method for the detection and quantification of sertraline, bearing in mind the available technical resources.

Secondly, the study and evaluation of direct photodegradation of sertraline under simulated sunlight and controlled laboratory conditions.

Thirdly, the search for indirect photodegradation of sertraline, also under controlled conditions, using organic matter (in the form of three types of natural humic substances, which by themselves have different characteristics at molecular level), ferric substances (in their natural form as well as in the presence of carboxylate groups, which might be a better representation of their effect in nature) and nitrates.

Finally, the analysis of photodegradation of sertraline using wastewater samples (wastewater influents and effluents from two wastewater treatment plants) and natural surface water matrices (fresh water from a river and brackish water from a tidal inlet of a lagoon), with the intent of verifying the behaviour of the pharmaceutical and attempting to shed some light on the most influencing factors.

Chapter 2 Materials and Methods

2.1 Reagents and solvents

Sertraline Hydrochloride, $C_{17}H_{17}Cl_2N.HCl$ (>98.0%), from Tokyo Chemical Industry Co, Ltd; Humic substances (fulvic acids, humic acids, XAD-4 fraction), extracted from water samples from Poço da Cruz by Esteves (1995); Sodium oxalate, $C_2O_4Na_2$ (>99%), from May & Baker, Ltd, Dagnham, England; Iron(III) Chloride 6-hydrate, $FeCl_3.6H_2O$, from DIDACTIC Panreac Química SA, Spain; Sodium Nitrate, $NaNO_3$ (99.0%), from Panreac Química SA, Spain.

Acetic Acid, $C_2H_4O_2$ (100%) (GR for analysis), from MERCK; Acetonitrile, CH_3CN (99.9% - HPLC grade), from Chem-Lab NV, Belgium.

All solutions were prepared in ultrapure water (Milli-Q grade).

2.2 Collection and preparation of matrices

2.2.1 Natural matrices

The natural samples were collected from four different locations – two Wastewater Treatment Plants (WWTP), a river (Vouga River) and a lagoon (*Ria de Aveiro*), all located within the county of Aveiro (as represented in Figure 2.1).

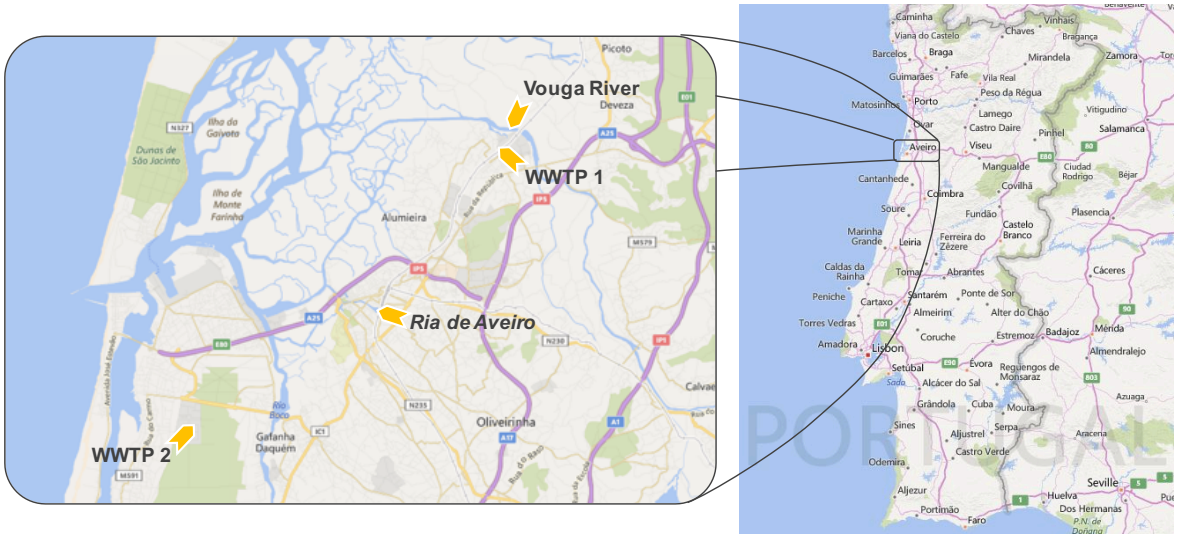

Figure 2.1 – Location of the sampling points.

Both WWTPs are located nearby industrial and residential areas and receive wastewater of both domestic and industrial sources. The wastewater is subjected to pre-treatment, primary settling tanks, biological treatment and secondary settling tanks, before being released back into the water

cycle. In each of the WWTPs, the samples were collected from two points – after the primary settling tanks and after the biological treatment. The primary treatment acts mainly on the reduction of suspended solids, as well as also reducing the BOD (Biochemical Oxygen Demand) concentration. The secondary treatment acts mainly on the dissolved organic matter, through biological processes that break it down into carbon dioxide, water and energy, resulting in an effluent with much lower BOD content and suspended solids. Thus, these differences result in effluents with very distinct composition which might result in different photodegradation phenomena, which will be evaluate throughout this study.

The Vouga River is born in district of Viseu and its river basin covers the greatest area of Aveiro district. The *Ria de Aveiro* is also integrated within the Vouga river basin and consists of an estuarine lagoon that spreads over several channels, and the sample was collected at the endpoint of one of the channels, inside the city of Aveiro.

Table 2.1 summarizes the water samples, the locations and dates of collection.

Table 2.1 – Locations and dates of collection of natural samples.

Sample name	Collection date	Location	Sample treatment
WWTP1	16/03/2015	WWTP 1 – after primary treatment	Filtration through 0.45 μm filter and refrigeration at 4°C
WWTP1-PT	25/06/2015	WWTP 1 – after primary treatment	Filtration through 0.22 μm filter and refrigeration at 4°C
WWTP1-ST	08/06/2015	WWTP 1 – after secondary treatment	
WWTP2-PT	08/06/2015	WWTP 2 – after primary treatment	
WWTP2-ST	08/06/2015	WWTP 2 – after secondary treatment	
RA	12/07/2015	*Ria de Aveiro*	
VR	25/06/2015	Vouga River	

The last column of Table 2.1 concerns the type of treatment that the sample was subjected prior to the photodegradation tests. The irradiation procedures (further explained in subchapter 2.3.2) can reach temperatures between 30 and 45 °C, as the solar irradiator's fans (air refrigerated system) can only keep the temperature down to a certain extent. Coincidently, the optimal temperature for the growth of bacteria falls under this range (for instance, mesophilic bacteria live and grow in temperatures from 20 up to 50 °C (Metcalf & Eddy 2003)). This microbiological activity might lead to biodegradation of the pharmaceutical, an undesirable effect for the purpose of this study. Accordingly, to avoid biodegradation which would cause misleading results, the natural matrices were initially filtered through a 0.45 μm porosity filter. However, during the first attempt of

irradiation with a natural matrix (WWTP1), the dark controls displayed considerable degradation of sertraline, which had not been detected during the irradiations with synthetic matrices, so this was suspected to be connected to biological activity. For this reason, the subsequent matrices were filtered through a 0.22 μm porosity filter (which provides a more efficient filtration of bacteria and other microorganisms (Shirey & Bissonnette 1991)).

2.2.2 Synthetic matrices

The synthetic samples were prepared by dissolving the pharmaceutical compound and the photosensitizer in ultrapure water, aiding the dissolution with an ultrasound bath when needed. The tested photosensitizers (Table 2.2) were selected according to photodegradation studies carried out for other contaminants or pharmaceutical compounds (Calza *et al.* 2012; Chen *et al.* 2013; Lee *et al.* 2014). The influence of oxygen was tested by sparging solutions of sertraline with O_2 (for the presence of oxygen) and with N_2 (for the absence of oxygen).

Table 2.2 – Concentrations of each photosensitizing agent tested during the irradiation procedures.

Photosensitizing agent		Concentration [mg/L]
Dissolved Organic Matter	Fulvic Acids (FA)	1 ; 5 ; 13 ; 20 ; 21 mg/L FA
	Humic Acids (HA)	1 ; 5 ; 13 ; 20 mg/L HA
	XAD-4 fraction	1 ; 5 ; 13 ; 20 ; 25 mg/L XAD-4
Fe(III)	$FeCl_3.6H_2O$	1 ; 3 mg/L $FeCl_3$
Fe(III)-oxalate	$FeCl_3.6H_2O$ and $C_2O_4Na_2$	3 mg/L $FeCl_3$ and 20 mg/L $C_2O_4Na_2$
Nitrate	$NaNO_3$	13 mg/L $NaNO_3$
Oxygen	Sparge with O_2 and N_2	-

2.3 Photodegradation experiments

2.3.1 Equipment and theoretical background

The photodegradation experiments were carried out on a SolarBox 1500 (Co.fo.me.gra., Italy), equipped with a Xenon lamp (1500 W) and UV radiation filters which restrict the transmission of light for wavelengths below the 290 nm. The lamp was kept at a constant intensity of 55 W/m^2 in the range of 290 – 400 nm (or 550 W/m^2 in the range of 290 – 800 nm), and the temperature and irradiation intensity were controlled using a multimeter (Co.fo.me.gra., Italy), respectively, with a black standard temperature sensor and a UV 290 – 400 nm large band sensor.

Figure 2.2 represents the spectra of the Xenon lamp and of the Sun, calculated for the irradiance as it is received at the surface of Earth. Despite the differences in some areas of these spectra, it is a

fair representation of the natural sunlight, when compared to other lamps such as UVA or UVB fluorescent lamps.

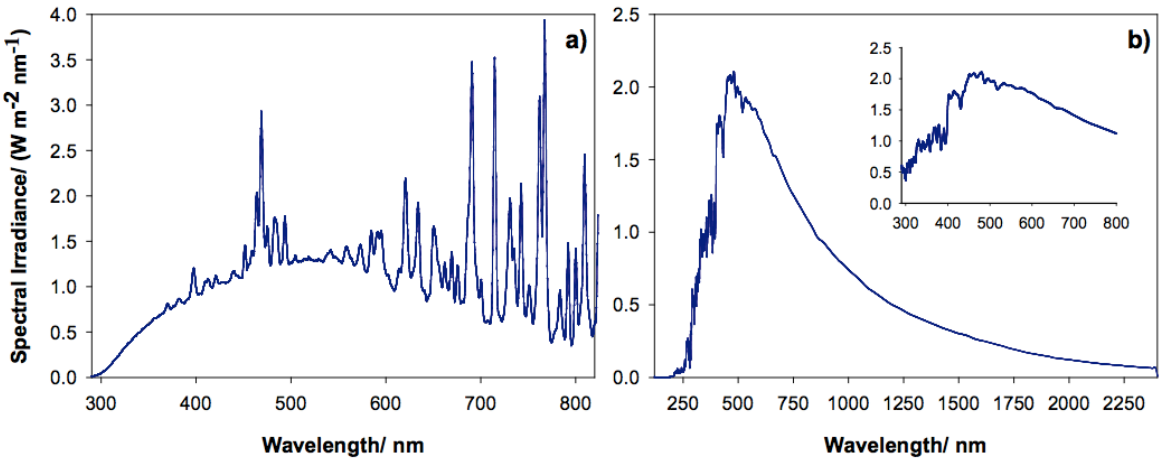

Figure 2.2 – a) Spectral irradiance of a 1500 W arc xenon lamp when using an outdoor UV filter, as given by the manufacturer (Solarbox 1500, Co.fo.me.gra, Italy). The spectrum is referred to a total irradiance of 550 W m^{-2} between 290-800 nm. b) Solar spectral irradiance obtained under the SORCE project (NASA 2008). The spectrum is referred to 23rd July 2008; the values were averaged in order to provide daily solar irradiance (Calisto 2011).

In order to work around the more noticeable differences in the light spectrum and standardize the irradiation time to the amount of days that the photodegradation would take during actual irradiation under real sun light, these were converted to Summer Sunny Days (SSD), according to the following approach, suggested by Calisto *et al.* (2011). The conversion of irradiation times under simulated conditions to SSD is based on the measurements made by Vione *et al.* (2006) and Minero *et al.* (2007) where, during a cloudless summer day (July 15th) at a latitude of 45°N (Portugal's latitude is roughly 40°N), the total energy reaching the ground is 7.5×10^{5} J/m^{2} (290-400 nm), measured with the same multimeter that was used in the present study. Considering that the total energy provided by the Solarbox, measured by the multimeter, is 55 W/m^{2} (290-400 nm), then the total energy reaching the ground during a summer sunny day (24h day/night cycle) is equivalent to 3.8 h of irradiation under the artificial light provided by the Solarbox.

The use of this equipment for the simulation of solar irradiation instead of a direct exposure to sunlight concerns the actual benefits that arise from performing such experiments under controlled laboratory conditions. The temperature and irradiation can be kept constant and may be monitored, avoiding the influence of temperature fluctuations and other weather factors like cloudiness. Additionally, an irradiation apparatus allows for a continuous irradiation, whereas natural exposure to sunlight implies interruptions due to the natural day/night cycle.

2.3.2 Irradiation procedure

The photodegradation experiments were sorted into two categories, long irradiations and single time irradiations, which main purposes differed and are presented in Table 2.3.

Table 2.3 – Characteristics of each type of irradiations' experiments performed.

	Type of irradiation	
	Long irradiations	**Single time irradiations**
Amount/concentration of matrix	Only one concentration per matrix	Several concentrations tests for each matrix (*e.g.*, each irradiation experiment was performed with a different concentration of the matrix)
Duration of irradiation	Continuous irradiation at 55 W/m^2 (290-400 nm) with sampling, until, at least, half-life time is reached	30 h of irradiation at 55 W/m^2 (290-400 nm)
Objective	To determine the kinetic constant and the half-life time of the pharmaceutical in each matrix	To ascertain the influence of each photosensitizer and matrix in the photodegradation of the pharmaceutical

All irradiations with the different natural and synthetic matrices were performed at an initial concentration of 5 mg/L SER.HCl. Table 2.4 presents an overview of all the irradiations performed and the concentrations of the individual photosensitizer or of the natural water in each of them.

Table 2.4 – Matrices used for each type of irradiation and corresponding concentrations. See Table 2.1 for samples' names.

Long irradiations		**Single time irradiations**	
Matrix	**Concentration**	**Matrix**	**Concentration**
WWTP1-PT	98% (v/v)	WWTP1	10% ; 25% ; 50% ; 90% (v/v)
WWTP1-ST	98% (v/v)	Fulvic acids	1 ; 5 ; 13 ; 20 ; 21 mg/L
WWTP2-PT	98% (v/v)	Humic acids	1 ; 5 ; 13 ; 20 mg/L
WWTP2-ST	98% (v/v)	XAD-4 fraction	1 ; 5; 13; 20 ; 25 mg/L
RA	98% (v/v)	Fe(III)-Oxalate	0.6 mg/L Fe(III) and 13 mg/L C_2O_4
VR	98% (v/v)	Fe(III)	0.6 mg/L Fe(III)
Fulvic acids	20.1 mg/L	Nitrate	10 mg/L NO_3^-
Humic acids	12.6 mg/L	Oxygen	Sparge with O_2 and N_2
XAD-4 fraction	20.0 mg/L		
Fe(III)	0.2 mg Fe(III)/L		

During each irradiation experiment, a total of 3 samples and 3 dark controls (in quartz tubes) were placed inside the Solarbox, according to the scheme presented in Figure 2.3. The UV sensor was placed in the centre and the temperature sensor was placed on the left side of the box, under the dark control tubes (as these do not need to be perfectly irradiated and the presence of this sensor will not affect the performance of the irradiation). The rest of the tubes were placed throughout the apparatus and these positions remained the same for all irradiations.

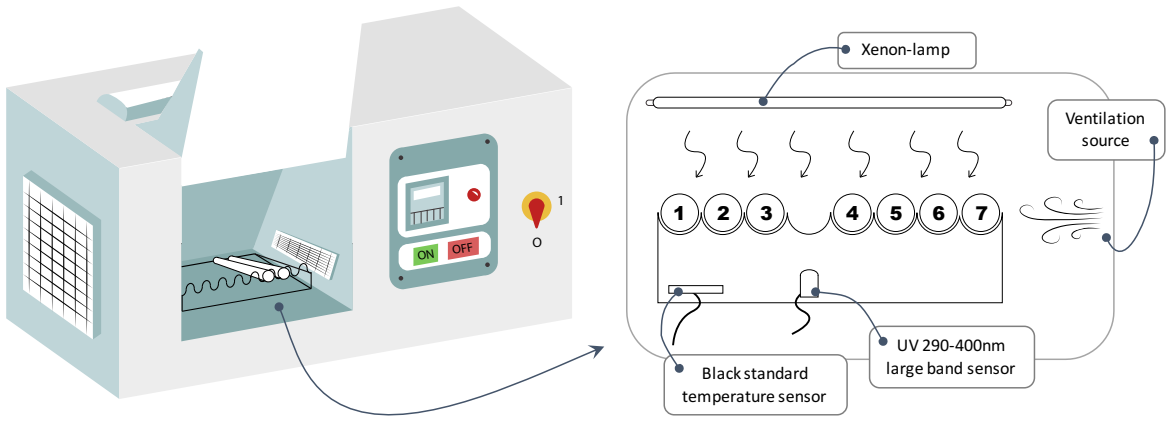

Figure 2.3 – Scheme of the irradiation apparatus. Quartz tube legend: 1 – dark control #1; 2 – dark control #2; 3 – irradiated sample #1; 4 – irradiated sample #2; 5 – irradiated sample #3; 6 – dark control #3; 7 – matrix without sertraline

Given the lack of knowledge concerning the composition of the natural matrices, when irradiating these matrices, an extra tube was irradiated, containing merely a sample of the matrix, without the addition of sertraline. This was done in order to rule out possible peaks which result from the matrix that could arise during the HPLC analysis of the samples that would otherwise be confused with the photodegradation products.

2.3.3 Kinetic modelling

The concentrations registered throughout each irradiation experiment underwent some transformations in order to account for the variations of concentration in the dark controls. The first step was to calculate the fraction of degradation of each irradiated sample and dark control ($Deg_{t,i}$):

$$Deg_{t,i} = \frac{C_0 - C_{t,i}}{C_0} \quad \text{Eq. 2.1}$$

Where C_0 corresponds to the concentration at t_0 moment of irradiation (as measured by the HPLC-UV) and $C_{t,i}$ to the concentration of replicate *i* at *t* time of irradiation, both in mg/L SER.HCl.

The second step consisted of the calculation of the photodegraded fraction of each sample ($Fot_{t,i}$), considering the average degradation of the dark controls:

$$Fot_{t,i} = Deg_{S,t,i} - Deg_{C,t} \quad \text{Eq. 2.2}$$

Where the subscript *S* corresponds to the irradiated samples and *C* to the dark controls (both $Deg_{S,t,i}$ and $Deg_{C,t,i}$ are calculated by Eq. 2.1)

Thirdly, the initial concentrations were corrected, accounting for the photodegraded fraction ($Fot_{t,i}$):

$$C'_{t,i} = C_0 \times \left(1 - Fot_{t,i}\right) \quad \text{Eq. 2.3}$$

To simplify the reading and comparison of data, the graphics presented throughout this study were normalized by the initial concentration ($C'_{t,i}$ / C_0).

After an initial study on the behaviour of SER under irradiation (further analysed in subchapter 3.3), it was verified that it follows a pseudo-first order rate, expressed mathematically as Eq. 2.4:

$$\frac{d[A]}{dt} = -k[A][B] = -k_{obs}[A] \quad \text{Eq. 2.4}$$

$$k = k_{obs}[B]_0 \quad \text{Eq. 2.5}$$

Where [A] consists of the concentration of the studied specie and [B] the concentration of other specie(s) that may take part in the reaction at a molecular level, but whose mechanisms are unknown. Hence, it is assumed that [B] remains constant (Eq. 2.5); therefore, *k* (first order rate constant, in h^{-1}) is replaced by k_{obs} (pseudo-first order rate constant, in h^{-1}), commonly referred to as observed rate constant (Schwarzenbach *et al.* 2003).

Eq. 2.6, obtained by the integration of Eq. 2.4, is used for the calculation of k_{obs} of each kinetic study and the respective half-life times. The values used for *C* in Eq. 2.6 are the values $C'_{t,i}$ presented in Eq. 2.3 which, throughout the study are simply represented as *C*. The data was adjusted to the kinetic model using the software Graphpad Prism 5 for the non-linear adjustment of the data to the model.

$$C = C_0 \times e^{-k_{obs}t} \quad \text{Eq. 2.6}$$

By definition, the half-life time ($t_{1/2}$) of a given substance consists of the time it takes for the substance to reach half of its initial concentration. In this particular type of reaction, sertraline's half-life is calculated with the estimated k_{obs} in each matrix, by Eq. 2.7. This value corresponds to hours of irradiation within the solarbox and was converted to SSD (previously explained in subchapter 2.3.1) with Eq. 2.8.

$$t_{1/2} = \frac{\ln 2}{-k_{obs}} \quad \text{Eq. 2.7}$$

$$t_{1/2}(SSD) = t_{1/2}(h) \times \frac{55\left(J/_{s.m^2}\right) \times 3600\left(s/_h\right)}{7.5 \times 10^5 \left(J/_{m^2}\right)} \quad \text{Eq. 2.8}$$

2.4 Analytical procedures

2.4.1 High Pressure Liquid Chromatography for quantification of sertraline

2.4.1.1 Equipment and theoretical background

Chromatography consists of a technique through which the components in a mixture can be separated, allowing for posterior identification, by making a mobile phase (which transports the mixture that needs to be separated) to pass through a stationary phase. The stationary phase may take different forms, depending on the desired effect.

The separation by chromatography depends on the type of molecules or compounds and their behaviour. As such, there are several types of separation methods that rely, for instance, on the adsorption affinity between the components and the stationary phase (adsorption chromatography), the differences in solubility (partition chromatography), the differences of polarity of each component in the mixture (hydrophobic interaction chromatography), the separation of ions and polar molecules depending on their affinity to the ion (ion-exchange chromatography), amongst others.

The type of chromatography explored during this work relies on the relative polarities of the mobile phase and the stationary phase. Within this type of chromatography is the method called High-Performance Liquid Chromatography (HPLC), also known as High-Pressure Liquid Chromatography. As its name suggests, this system relies on very high pressures that force the mobile phase through a tightly packed column (which is typically composed by silica supporting the stationary phase, but there are also other materials), allowing for more efficient separation of components and, therefore, has a higher sensitivity than simpler chromatography methods.

Within this system, there are normal-phase HPLC and reverse-phase HPLC. Both have the same mode of action, but normal-phase consists of a polar stationary phase and a non-polar mobile phase, whereas reverse-phase consists of a non-polar stationary phase (which might still be silica, a polar material, but has chains of carbons – usually C8 or C18 – attached that make it non-polar) and a polar mobile phase. The latter is the one used in this study.

Given the sensitivity of these columns, they are often used in conjunction with a guard column that should have the same composition of the main column, but may have a higher porosity and higher packing particles size. Basically, guard columns act as a protection of the analytical column, extending its lifetime.

Put simply, in the case of a reverse-phase, if an analyte is non-polar, then it will have a higher affinity for the column, and will be retained for a longer time. In order to have some control over the separation of components in a sample, the polarity of the mobile phase can be adjusted by using a mixture of, for instance, an aqueous phase (which is highly polar), and a solvent like methanol or acetonitrile (which are less polar, but not as much as the stationary phase), and altering the percentage of each until all the components are separated and within a reasonable separation time.

After the separation, the compounds need to be detected, resulting in a chromatogram, and this also depends on the properties of the sample and/or the components to be separated. There are UV/Vis detectors (for substances that absorb light in the UV or in the visible wavelength range), diode-array detectors (also for substances that absorb in the UV-range, but can deliver a full spectrum throughout the separation), fluorescence detectors (for substances that fluoresce, and which is particularly interesting for its high sensitivity), electrical conductivity detectors (for ionic components), amongst others. As the separation occurs, the detector starts converting the analyte detected into a signal that a computer may recognize.

In brief, a HPLC requires the reservoirs for the mobile phase, the high pressure pump, the sample valve, the column, the detector and a computer for data acquisition and data processing (see diagram in Figure 2.4). The equipment used throughout this study consisted of a Shimadzu Prominence HPLC, equipped with a solvent delivery unit (model LC-20AD), a degasser (model DGU-20A_5), a UV/Vis detector (model SPD-20A), and a column oven (model CTO-10AS VP), holding an ACE®-PFP-C18 column (150 mm x 4.6 mm inner diameter, 5 μm) and an ACE-C18 guard column. The whole system can be controlled via the software LcSolutions, and the data is processed by software LabSolutions, both provided by Shimadzu.

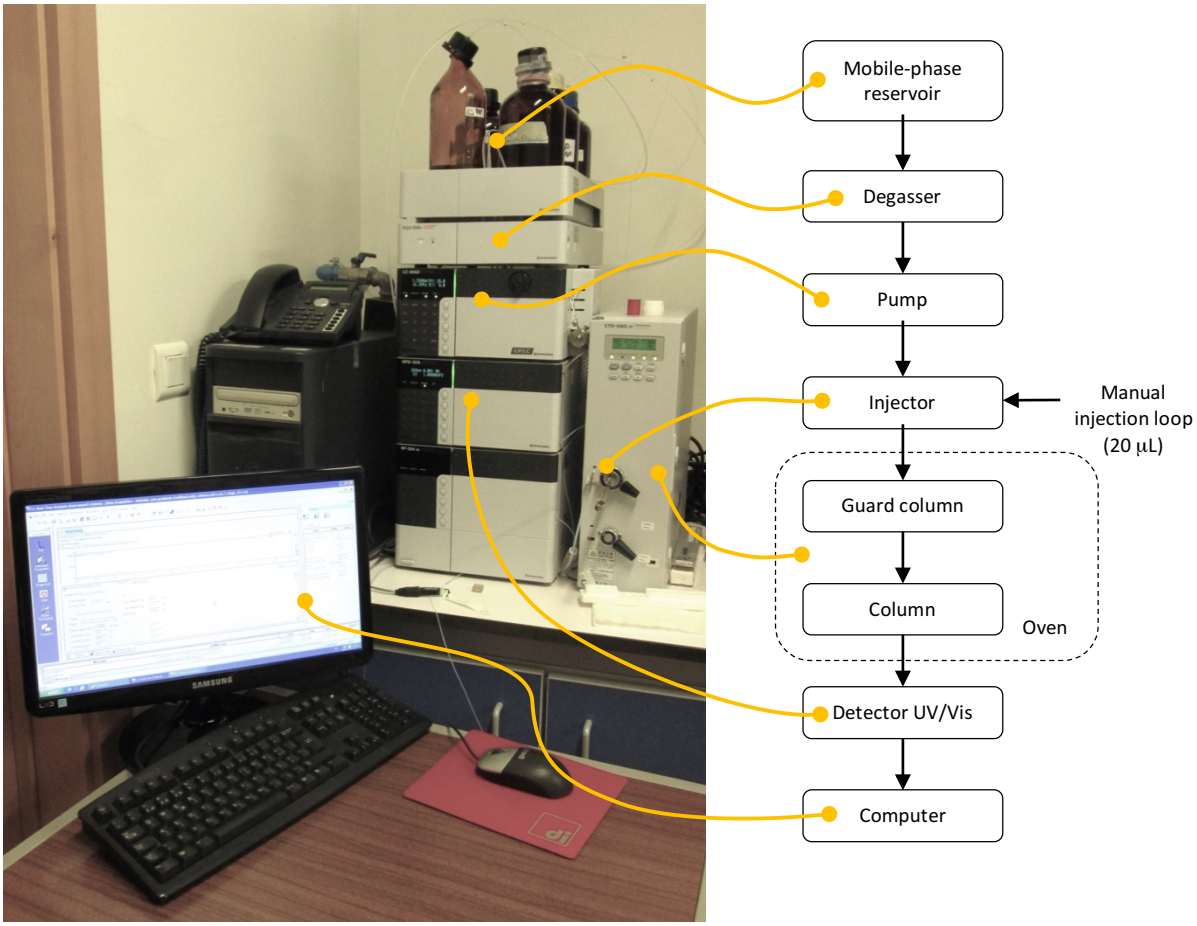

Figure 2.4 – Configuration of the HPLC-UV equipment used for the analysis of samples throughout the study.

Ideally, peaks should have a Gaussian shape and this can be achieved through a study of the best type of column for the compound, the flow, the mobile-phase composition, the oven temperature and the injection volume.

Some parameters are inherent to every chromatogram, as is exemplified in Figure 2.5, among which stand out the void time (t_m, consists of the time it takes for the molecules of the injected sample to travel through the column without being retained by the stationary phase, which commonly happens to the solvent of the sample), the retention time (t_r, is the time that the analyte takes to travel through the stationary phase), the time in stationary phase (t_s, consists of the difference between the retention time and the void time), the height of the analyte peak (h) and the peak area (A) (McMaster 1994).

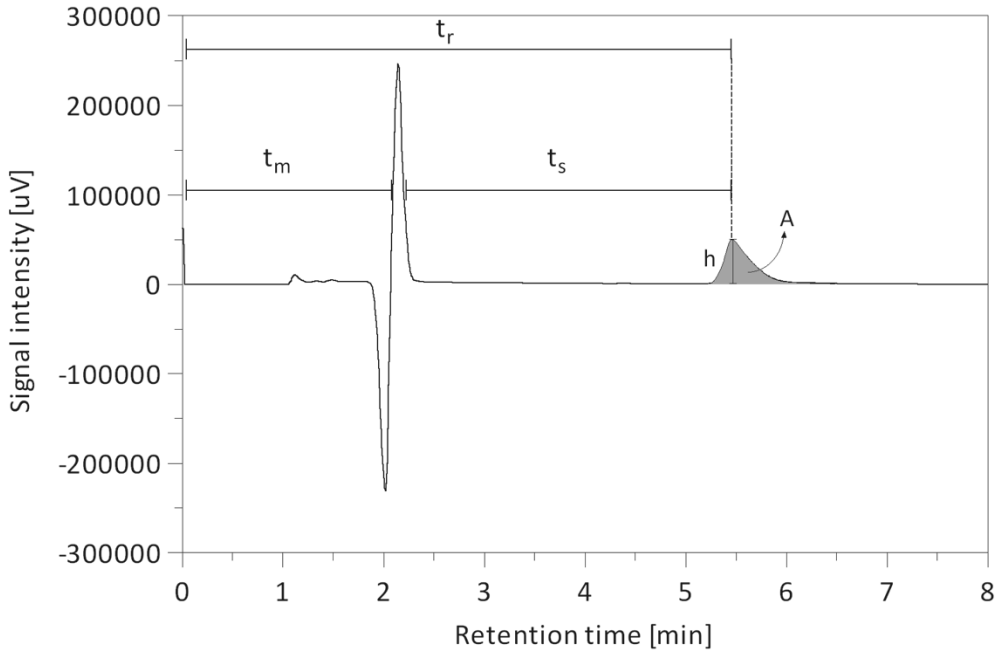

Figure 2.5 – Example of a chromatogram of an aqueous solution of sertraline (5.0 mg SER.HCl/L). Legend: t_r: retention time, t_m: void time, t_s: time in stationary phase, h: peak height, and A: peak area.

The first peak (after the void time) is a result of the differences of refraction index between the aqueous phase of the injected sample and the mobile phase, and appears always at the same time as it is mainly related to the column length and the flow.

The peak height and peak area may be used for the quantification of the studied substance or compound. The peak area is often chosen (in *lieu* of the peak height) to build the calibration curve, as it is more reliable and reproducible (especially in the case of a non-symmetric peak). The calibration curve comes as a function of peak area by standard concentration:

$$Area = a + b \times Concentration \qquad \text{Eq. 2.9}$$

2.4.1.2 Calibration and statistical analysis

Considering that the photodegradation studies were intended to start with a concentration of 5 mg/L SER.HCl and in order to follow until at least 95% of its degradation, the calibration curve for the HPLC should be in the range of 0.25 and 5 mg/L SER.HCl. Accordingly, a stock solution (5 mg/L SER.HCL) was prepared by the dissolution of the substance in ultrapure water, aided by an ultrasound bath, and seven standards were prepared with the following concentrations: 0.25, 0.50, 1.00, 2.00, 3.00, 4.00 and 5.00 mg SER.HCl/L.

Each standard was acidified to a pH between 3.0 and 4.0 with acetic acid. This was done due to the inexistence of a linear response between peak area and concentration for non-acidified standard solutions (Figure 2.6). Accordingly, each sample was acidified with the necessary volume of acetic acid to achieve a pH between 3.0 and 4.0 prior to injecting to the HPLC-UV/Vis.

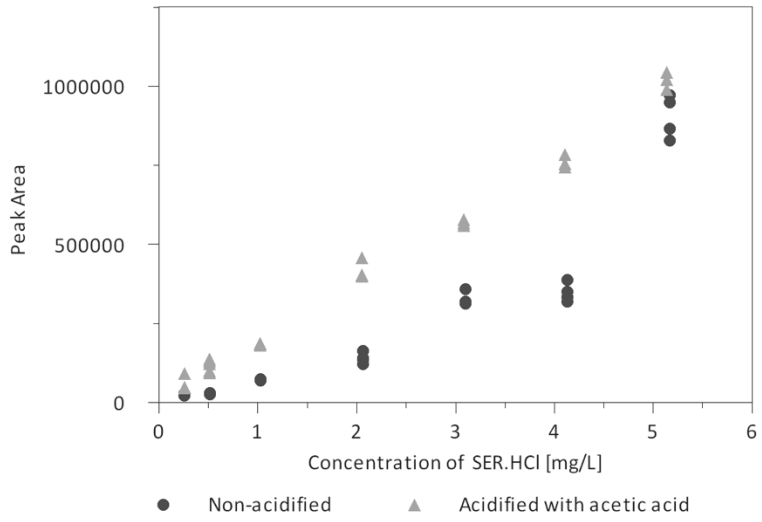

Figure 2.6 – Differences between the correlation of peak areas and the concentration of acidified standard solutions and non-acidified standard solutions.

The highest and lowest standard solutions were injected daily to assure the validity of the last calibration procedure. If the deviation between the new standards' peak areas and the corresponding peak areas (calculated by the latest curve) were significant (deviations greater than 10% in the lowest standard and 5% in the highest standard), then a new calibration procedure was performed.

The statistical analysis of each calibration curve was done according to Miller & Miller (2005) and ICH (1994), as well as the calculations for the limits of detection (Eq. 2.10) and of quantification (Eq. 2.11):

$$LOD = \frac{3.3 \times \sigma_a}{b} \quad \text{Eq. 2.10}$$

$$LOQ = \frac{3.3 \times \sigma_a}{b} \quad \text{Eq. 2.11}$$

Where the value σ_a corresponds to the standard deviation of y-intercepts of the regression line and *b* to the standard deviation of the slope.

2.4.2 Mass spectrometry for identification of photoproducts

The photoproducts formed through direct photodegradation (irradiation of sertraline in ultrapure water) were analysed by mass spectrometry. Samples from the both the irradiated tubes and the control tubes were collected after 100 hours of irradiation. The selection of the irradiation time was defined by the moment at which the photoproducts detected through HPLC-UV were at its highest peak area, in order to guarantee a good signal from each photoproduct. A standard solution of sertraline was also analysed. Each sample was diluted in methanol (0.1% formic acid v/v).

The identification of photoproducts was carried out with a Micromass Q-TOF2 hybrid tandem mass spectrometer (Manchester, UK), operated in positive-ion mode. The operating conditions for ESI-MS were as follows: flow rate of 0.10 μL/min, Time-of-Flight mass resolution set to approximately 9000, cone voltage of 35 V, capillary voltage of 3 kV, source temperature of 80 °C and desolvation temperature of 150 °C. ESI-MS^2 spectra were obtained using argon as collision gas and the collision energy varied between 10 and 35 eV.

2.4.3 Quantification of Total Organic Carbon

The concentrations of total carbon (TC) and inorganic carbon (IC) were determined by means of a TOC analyser (TOC-VCPH, Shimadzu). The determination method (represented on Figure 2.7) consists on the conversion of all the TC components present in the sample into CO_2 by combusting it at 680°C. Afterwards the gases resulting from the sample (including CO_2) are cooled and dehydrated and stripped of chlorine and other halogens. The CO_2 is then detected in a Non-Dispersive Infrared Detector (NDIR) gas analyser, which outputs an analog signal that forms a peak.

The area of the peak and the corresponding concentration is then used to create the calibration curve.

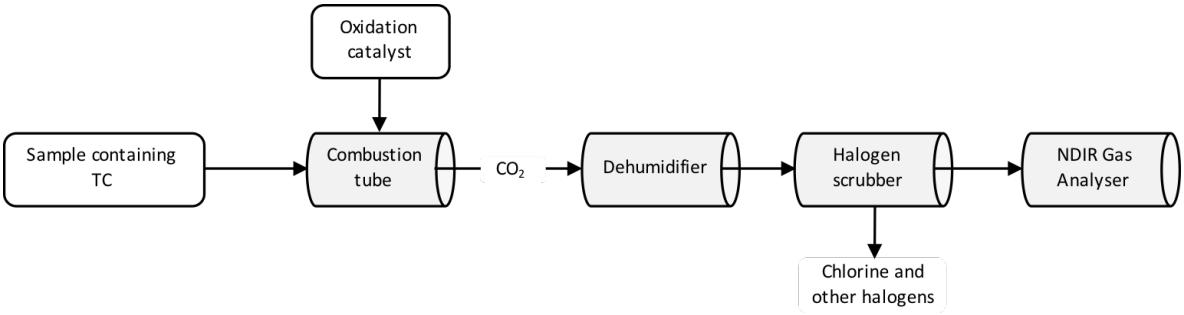

Figure 2.7 – Diagram of the procedure followed by the TOC Analyser.

The inorganic carbon consists of carbon derived from carbonates, hydrogen carbonates and dissolved carbon dioxide. These are converted to CO_2 by acidifying the sample with HCl:

$$Me_2CO_3 + 2HCl \rightarrow CO_2 + 2MeCl + H_2O \quad \text{Eq. 2.12}$$

$$MeHCO_3 + HCl \rightarrow CO_2 + MeCl + H_2O \quad \text{Eq. 2.13}$$

The CO_2 is then volatilized by sparging the sample with nitrogen and the rest of the procedure is identical to the TC procedure.

Normally, the total organic carbon (TOC) concentration is obtained by the difference between TC and IC, however, when the resulting TOC is higher than IC, then a different procedure should be adopted. The inorganic carbon present in the samples could be manually removed, by acidifying it with HCl and sparging the sample with nitrogen, and only then can the sample be reanalysed for TC (which now corresponds to TOC, since there is technically no IC). As the samples were filtered the TOC is the dissolved organic carbon (DOC) and the IC is the dissolved inorganic carbon (DIC).

The standard solutions for the calibration curves were prepared with Potassium Hydrogen Phthalate (KHP) for the TC calibration curve (in the range of 0.5 to 10 mg/L) and with Sodium Hydrogen Carbonate ($NaHCO_3$) and Sodium Carbonate (Na_2CO_3) for the IC calibration curve (in the range of 1 to 10 mg/L).

2.4.4 Quantification of Nitrates and Nitrites

The determination of the sum of nitrates (NO_3^-) and nitrites (NO_2^-) present in the samples was performed with a FIAstar 5000, according to the procedure described in ISO 13395-1996.

The method (represented in Figure 2.8) is based on the cadmium reduction method. The sample, containing NO_3^- and NO_2^- is injected through a cadmium reductor, which reduces the NO_3^- present in the solution to NO_2^-. This reduction causes an increase of pH, so the sample is initially mixed with a buffer solution (consisting of ammonium chloride). To the resulting solution, which now has only NO_2^-, a solution of acidic sulphanilamide is added to form a diazo compound. Sequentially, this compound reacts with NED (N-(1-naphtyl)-Ethylene-Diamine Dihydrochloride), resulting in a purple azo dye which is finally measured at 540 nm with a spectrophotometer.

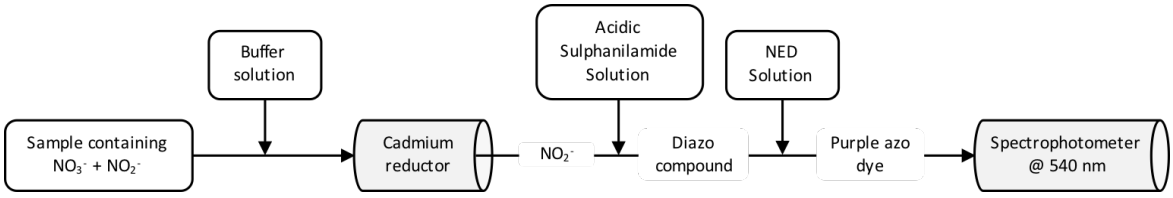

Figure 2.8 – Diagram of the procedure followed by the Nitrates and Nitrites Analyser.

The calibration solutions consist of Sodium Nitrate diluted in distilled water, and the calibration standards should be prepared in the concentration range of 0.1 to 5 mg/L NO_3 (40 μL loop), or the range of 0.005 to 0.25 mg/L NO_3 (400 μL loop).

2.4.5 Quantification of Iron species by Atomic Absorption Spectroscopy

The determination of iron was carried out using a Perkin Elmer AAnalyst 100 Atomic Absorption Spectrometer, according to the "Standard Atomic Absorption Conditions for Fe" provided with the equipment.

The atomic spectroscopy consists of the vaporization of a sample at very high temperatures (2100-2300 °C) to decompose it into atoms. The absorbance of these atoms at the wavelength corresponding to the element to be analysed is then measured and converted to a concentration by means of a calibration curve. The quantification of iron was carried out at a wavelength of 248.3 nm, and because of the low concentration of iron in the samples, a scale expansion (6) was used in order to improve the equipment's sensitivity.

Calibration standards were prepared in the range of 0.05 to 0.60 mg/L of Fe from dilutions of a standard solution (1000 mg/L Fe) prepared according to the equipment's protocol.

2.4.6 UV/Vis absorption spectra

The measurement of the ultraviolet/visible spectra of each of the matrices and sertraline in ultrapure water were carried out with the spectrophotometer T90+ (PG Instruments), and a quartz cell with an optical path of 1 cm was used. The absorptivity spectra were calculated from these measurements, using the Beer-Lambert Law:

$$Abs = \varepsilon \times C \times l \qquad \text{Eq. 2.14}$$

Where *Abs* is the measured absorbance, ε is the molar absorptivity coefficient ($mol^{-1}.L^{-1}.cm^{-1}$), *C* is the concentration of the analyte (mol/L) and *l* is the optical path length (cm).

Chapter 3 Results and Discussion

Optimization and performance of the HPLC-UV method

Characterization of matrices

Direct photodegradation of sertraline in ultrapure water

Photodegradation of sertraline in natural matrices

- Wastewater treatment effluent matrices
- Superficial water matrices
- Dilution of WWTP's effluent matrix

Influence factors on photodegradation of sertraline

- Organic matter
- Ferric substances and Nitrates
- Presence/Absence of oxygen

Photosensitizing effect in natural and synthetic matrices

Detection of photoproducts by HPLC-UV

Identification of photoproducts by mass spectrometry

3.1 Optimization and performance of the HPLC-UV method

The studies reported in the literature for the quantification of sertraline presented in the subchapter 1.5 served as a guide for the development and optimization of the analytical method (HPLC-UV).

The wavelength used in the UV detector was determined based on the UV spectrum of sertraline in ultrapure water (Figure 3.1). The spectrum revealed a local maximum (at 273 nm) but with a very low absorptivity. The biggest absorptivity is in the wavelength range of 200-240 nm, but the signal might be very unstable because a small shift in wavelength causes a bigger deviation in the signal.

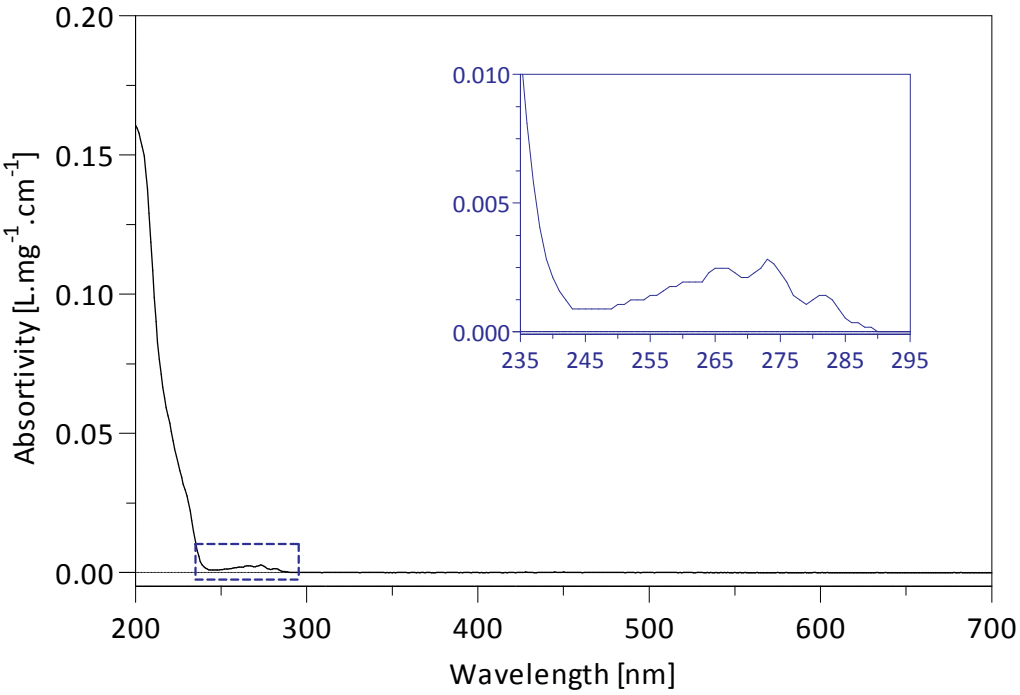

Figure 3.1 – Absorptivity spectrum of SER.HCl in ultrapure water, obtained according to the procedure described in subchapter 2.4.6.

However, a compromise was taken between a higher sensitivity and lower precision by choosing the wavelength of 210 nm.

The chosen mobile phase was acetonitrile (given its predominance in the literature, evident from Table 1.5) and water acidified to a pH of 3.0-3.4 with acetic acid, and the flow was 0.9 mL/min which lead to a sufficiently low back pressure (to avoid damaging the column).

The percentages of each solvent were selected so as to combine low separation time with high resolution between sertraline and its photoproducts (generated during irradiation), after testing the following combinations:

- 50% ACN and 50% H_2O: These fractions resulted in a peak at 2.317 minutes, too close to the void time (t_m). A blank sample (H_2O) was injected to confirm that the observed peak was sertraline.
- 30% ACN and 70% H_2O: The peak was considerably delayed, appearing at 18.207 minutes. This means that the substance has a big affinity to the stationary phase, and the mobile phase is too polar (because of the high fraction of water), so the fraction of acetonitrile (less polar) needs to be increased.
- 45% ACN and 55% H_2O: The peak appears at 2.887 minutes. The photodegradation products might come within the first minutes so, as precaution, the retention time should be slightly longer, to avoid co-elution.
- 40% ACN and 60% H_2O: The peak has a retention time of 5.812 minutes, reducing the probability of co-elution.

Finally, in light of the results presented above, the analytical quantification of sertraline was carried out with an HPLC-UV (using an ACE®-PFP-C18 column (150 mm x 4.6 mm *i.d.*, 5 μm), and an ACE-C18 guard column) with a flow rate of 0.9 mL/min, oven temperature of 25°C, mobile-phase consisting of acetonitrile and water (acidified to pH 3.0 with acetic acid) (40:60, v/v), and injection volume of 20 μL.

Throughout the photodegradation studies, several calibration curves were performed. Figure 3.2 presents an example of a calibration curve and all the data processing that each calibration curve underwent.

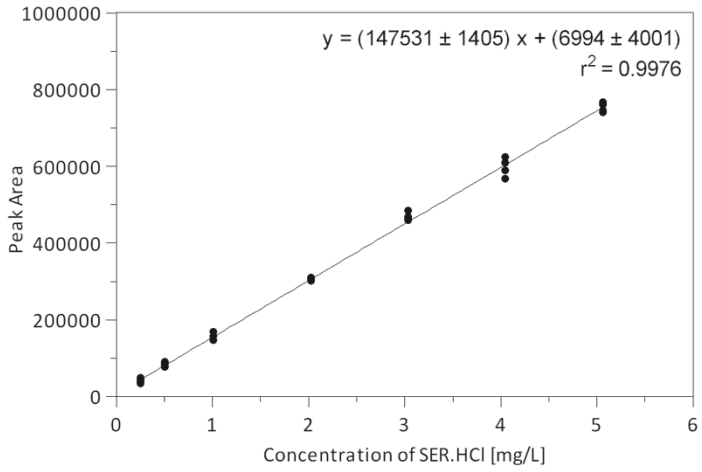

Figure 3.2 – Calibration curve for the quantification of SER.HCl by HPLC-UV (n=28).

The calculated limits of detection (LOD) and quantification (LOQ) were 0.085 mg/L and 0.258 mg/L SER.HCl, respectively. These values were obtained for the calibration curve presented in Figure 3.2. As already mentioned (see subchapter 2.4.1.2), the performance of the method was periodically tested in order to check the validity of the last calibration procedure. The average retention time (t_r), and respective standard deviation, for the detection of SER corresponded to 5.7 ± 0.5 minutes (n=147).

3.2 Characterization of matrices

The matrices used for photodegradation experiments were tested for the concentration of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), iron concentration (Fe), the sum of nitrates and nitrites (NO_3 and NO_2) and the pH value, all of which are presented in Table 3.1. There is a clear reduction of DOC of the WWTP matrices from secondary treatment, when compared to the primary treatment and the matrices from the natural superficial waters display a lower DOC content. The iron concentrations do not appear to show any relation to the source of the sample, however, the effluents from WWTP1 show a slightly higher concentration. The nitrates and nitrites of the WWTP samples are considerably low, compared to the natural superficial water samples.

Table 3.1 – Concentrations of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), iron (Fe), sum of nitrates and nitrites (NO_3 and NO_2) and pH value of each natural sample, and respective standard deviations.

Matrix*[1]	DOC ± σ [mg/L]	DIC ± σ [mg/L]	Fe ± σ [mg/L]	NO_3 and NO_2 [mg/L]	pH value
WWTP1	18.30 ± 0.08	116 ± 3	0.13 ± 0.01	0.018	8.15
WWTP1-PT	74 ± 2	148 ± 5	0.34 ± 0.01	0.018	8.11
WWTP1-ST	31 ± 1	85 ± 2	0.265 ± 0.009	0.042	8.14
WWTP2-PT	92.6 ± 0.4	87 ± 2	0.12 ± 0.02	0.031	7.55
WWTP2-ST	29.2 ± 0.1	83.1 ± 0.1	0.20 ± 0.01	0.007	8.84
RA	15 ± 1	31.8 ± 0.8	0.14 ± 0.01	0.709	7.46
VR	18.4 ± 0.8	17.7 ± 0.5	0.06 ± 0.01	0.373	8.33

*[1] See Table 2.1 for samples' names.

The absorbance spectra of the used matrices (Figure 3.3) do not appear to show significant differences apart from WWTP1-PT, which clearly absorbs more than the rest of the samples. This suggests that this sample may have more constituents (or lesser, but in higher concentrations) that are more susceptible to absorbing UV/Vis radiation.

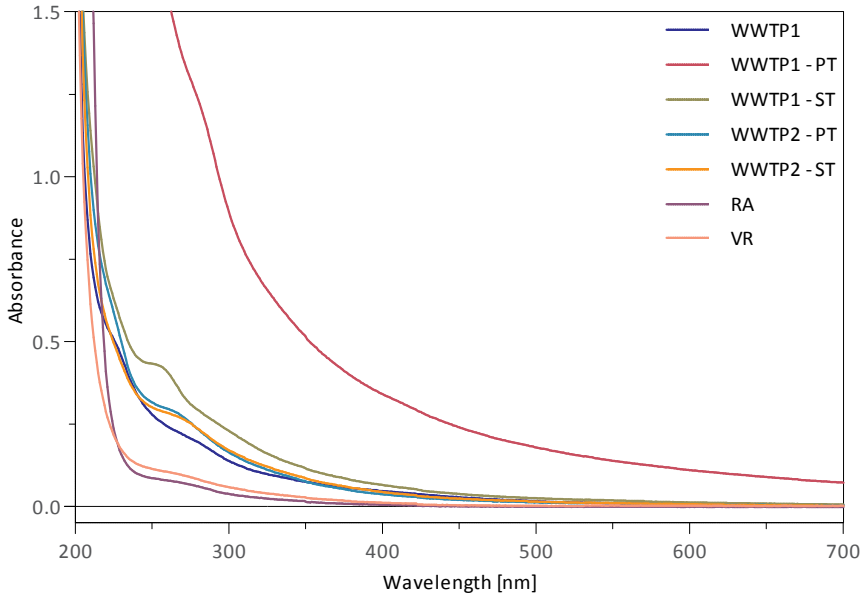

Figure 3.3 – UV/Vis absorbance spectra of the natural samples, obtained according to the procedure described in subchapter 2.4.6.

The synthetic matrices composed of humic substances do not contain either metallic substances, given their procedure of extraction which effectively removes all of these, neither nitrates and nitrites. Their main composition is organic carbon, measured according to the procedure in subchapter 2.4.3, and is presented in Table 3.2.

Table 3.2 – Concentrations of dissolved organic carbon (DOC) of synthetic matrices prepared with humic substances.

Matrix	DOC ± σ [mg/L] per 1 ppm HS	DOC ± σ [mg/L] *[1]
FA	0.522 ± 0.004	10.48 ± 0.09
HA	0.520 ± 0.003	6.53 ± 0.03
XAD-4	0.513 ± 0.003	10.26 ± 0.07

*[1] Concentrations used in the long irradiations.

The absorbance spectra of humic substances (see Figure 3.4) shows considerable differences between the three humic substances. The high absorbance of humic acids, compared to fulvic acids or XAD-4 fraction, shows that there is a higher presence of chromophores in the matrix (the main component in these types of matrices, composed of a high content of organic carbon) which suggests that there is a higher susceptibility to photodegradation. XAD-4, on the contrary, shows lower absorbance throughout the visible wavelength range, suggesting a potential lower susceptibility to photochemical reactions.

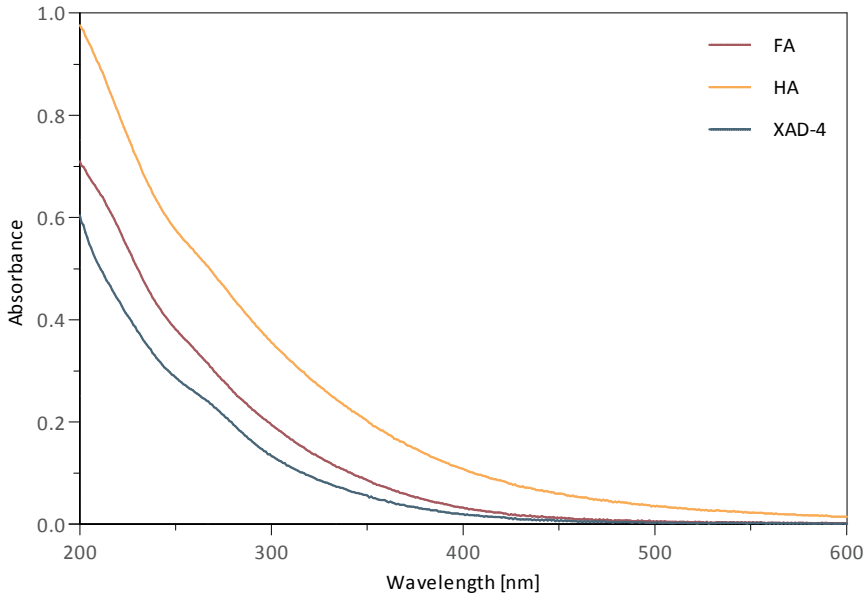

Figure 3.4 – UV/Vis absorbance spectra of the used humic substances (each substance at a concentration of 20 mg/L), obtained according to the procedure described in subchapter 2.4.6.

3.3 Direct photodegradation of sertraline in ultrapure water

In order to understand the direct photodegradation of sertraline under solar light radiation, an initial irradiation experiment was carried out for approximately 150 hours (Figure 3.5), with

sampling with intervals of 15 hours (aside from the last sampling which was longer to attempt reaching a lower concentration).

The process through which the data was processed involved the normalization of the concentrations of both irradiated samples and dark controls. In order to account for the variations that are not due to the radiation, the samples were corrected by the dark control concentration as explained in subchapter 2.3.3.

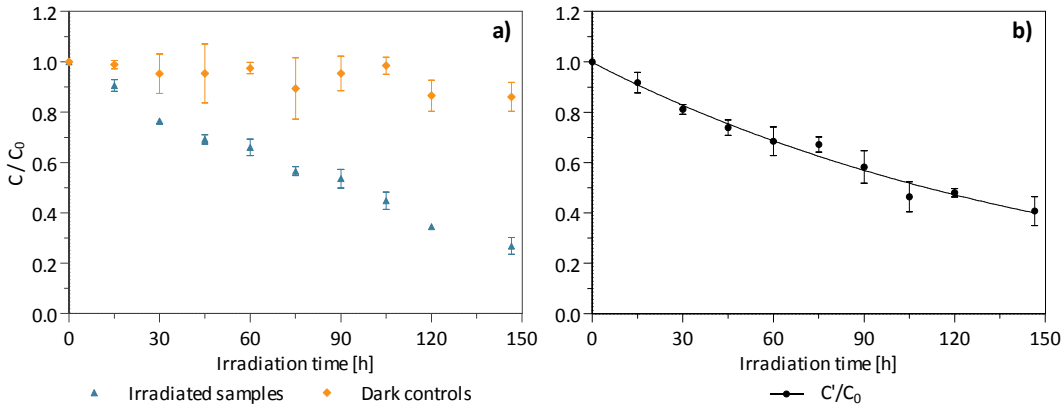

Figure 3.5 – a) Evolution of the concentration of SER.HCl in the irradiated samples and the dark controls and b) kinetic fitting to a pseudo-first order model of the photodegradation of SER.HCl in ultrapure water (after correction with dark controls). Each point (± standard deviation) represents the average of three replicates.

There are some fluctuations in the concentration of the dark controls that never exceed 20% of degradation, which, however, do not show an obvious decrease in the SER.HCl concentration, meaning that it can not be due to a degradation *per se* of SER.HCl, but to other phenomena. Accordingly, the variations were accounted for when calculating the photodegradation rate at each moment.

The resulting normalized concentrations (as seen in Figure 3.5b)), regarding solely the photodegradation effect (and rejecting any temperature effects or other influencing factors which would be accounted for in the dark controls) is satisfactorily described by a pseudo-first order kinetic model, with a determination coefficient (r^2) of 0.9498 (Table 3.3).

The calculated half-life time ($t_{1/2}$) is 111 ± 5 hours, which is equivalent to 29 ± 1 SSD (Summer Sunny Days), and comes to show that this compound, just by itself, is not easily degraded under solar light.

Table 3.3 – Observed photodegradation rate (k_{obs}), half-life time ($t_{1/2}$) in hours and in SSD for the modelling to a pseudo-first order kinetic for the photodegradation of sertraline in ultrapure water.

Matrix	*n*	r^2	$k_{obs} \pm \sigma$ [h^{-1}]	$t_{1/2} \pm \sigma$ [h]	$t_{1/2} \pm \sigma$ [SSD]
Ultrapure water	30	0.9498	0.0062 ± 0.0003	111 ± 5	29 ± 1

3.4 Photodegradation of sertraline in natural matrices

The photodegradation of sertraline was greatly enhanced in the presence of natural matrices, either from wastewater treatment plant effluents or from superficial water bodies. As mentioned before, all the irradiations started with an approximate concentration of 5 mg/L SER.HCl, with 3 irradiated tubes, 3 dark control tubes, and an additional tube solely with the matrix (to account for other unknown substances developing during the irradiation due to matrix components which would otherwise be considered photoproducts).

3.4.1 Wastewater treatment effluent matrices

Throughout the irradiations with natural samples spiked with SER, the dark controls from the matrices WWTP1-PT and WWTP2-ST presented little to no variation from the initial concentration. The other two matrices, WWTP1-ST and WWTP2-PT showed some irregular degradation but never exceeding a degradation of 20%. Regardless, the data was analysed similarly to the irradiation in ultrapure water, accounting for the registered variation of concentration in the dark controls at each sampling moment.

The irradiation with matrix from WWTP1-PT was unexpectedly faster than the irradiations in the other three matrices and was stopped at 45h since it already reached 90% of photodegradation.

There was a noticeable reduction in half-life times of sertraline in these matrices, all under 30 h (Table 3.4) in comparison with the direct photodegradation which took more than 100 h, pointing to a strong influence of indirect photodegradation phenomena.

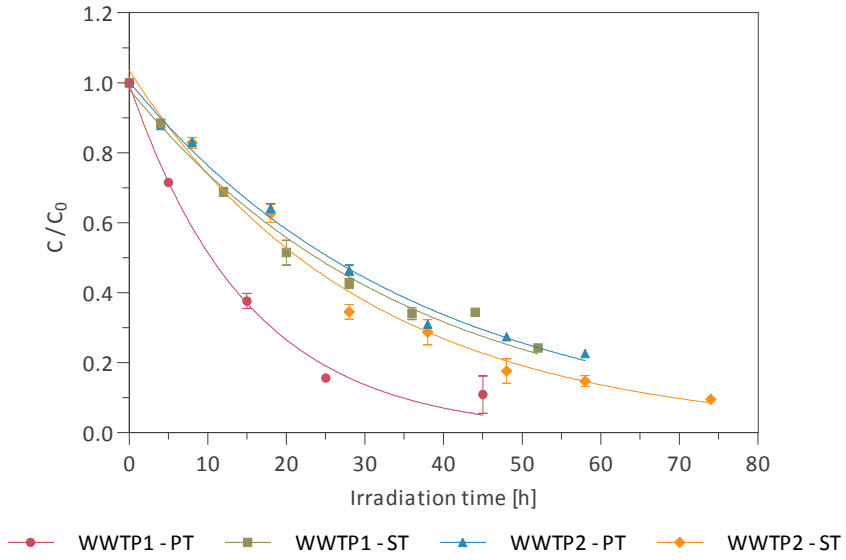

Figure 3.6 – Kinetic fitting to pseudo-first order model for the degradation of SER using matrices from wastewater treatment plants. Each point (± standard deviation) represents the average of three replicates.

There is not an obvious relationship between the concentration of DOC of each matrix and the observed half-life times: the DOC decreases from the primary to the secondary treatment (in both WWTPs), whereas the half-life times increase from primary to secondary treatments for WWTP1 and decreases for WWTP2 (Table 3.4).

The observed photodegradation rate obtained with samples from WWTP1 seem to increase with higher concentrations of iron, but the same phenomena is not verified in the irradiations with samples from WWTP2. It is possible that iron (although in small concentrations) may have contributed to the whole decrease in half-life times observed for these type of matrices, since it is a known photosensitizing agent, whose effect on the irradiations of sertraline is further studied in subchapter 3.5.2.

The UV/Vis absorbance spectra of these matrices show that WWTP1-PT has a considerably higher absorbance than the other three matrices (see Figure 3.3). This can be attributed to a higher vulnerability to photodegradation under solar light, thus it may act as a photosensitizer by transmitting the absorbed energy to sertraline, enhancing its degradation.

Table 3.4 – Observed photodegradation rate (k_{obs}), half-life time ($t_{1/2}$) in hours and in SSD for the modelling to a pseudo-first order kinetic for the photodegradation of sertraline in the wastewater treatment plant samples.

Matrix	*n*	r^2	$k_{obs} \pm \sigma$ [h^{-1}]	$t_{1/2} \pm \sigma$ [h]	$t_{1/2} \pm \sigma$ [SSD]
WWTP1-PT	15	0.9881	0.066 ± 0.003	10.5 ± 0.5	2.8 ± 0.1
WWTP1-ST	24	0.9791	0.028 ± 0.001	24.5 ± 0.8	6.5 ± 0.2
WWTP2-PT	24	0.9910	0.0273 ± 0.0007	25.4 ± 0.7	6.7 ± 0.2
WWTP2-ST	24	0.9766	0.034 ± 0.001	20.6 ± 0.9	5.4 ± 0.2

3.4.2 Superficial water matrices

The photodegradation differed slightly among the two superficial water matrices tested but overall, when compared to the WWTP matrices, the behaviour was relatively similar. Regarding the behaviour of the samples and dark controls throughout the irradiation, no significant deviation was registered between each irradiated sample and the dark controls did not present significant variations from the initial concentration (below 10%).

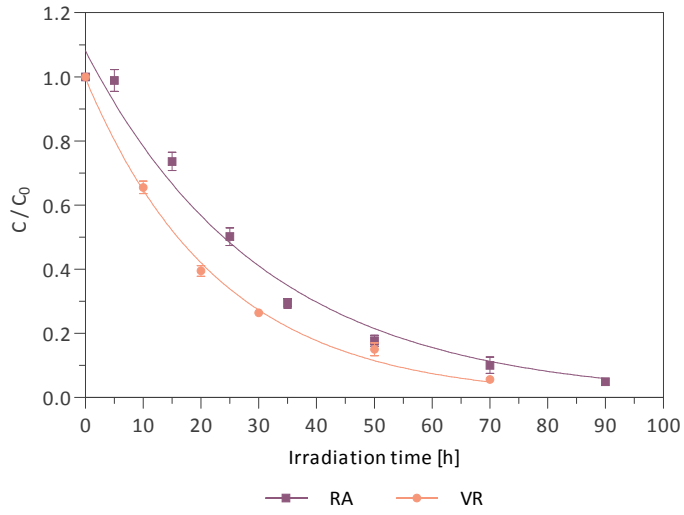

Figure 3.7 – Kinetic fitting to pseudo-first order model for the degradation of SER using matrices from the natural superficial waters (RA: *Ria de Aveiro*, VR: Vouga River). Each point (± standard deviation) represents the average of three replicates.

The irradiation carried out with Vouga River (VR) water lead to a higher photodegradation rate (lower half-life time), in relation to the the *Ria de Aveiro* (RA) water (Table 3.5). The iron and nitrates-nitrites concentrations in RA are almost double of those detected for VR matrix. Hypothetically, given these higher contents, it was expected that the irradiation in RA matrix would have contributed to a stronger formation of $O_2^{\bullet -}$, $HO^{\bullet}$ and OH^- (Eq. 1.9 to Eq. 1.14). Accordingly, assuming that sertraline is susceptible to the presence of these photosensitizers, a higher

photodegradation rate would be expected for the irradiation in RA matrix, which was not, as exposed above, verified.

Table 3.5 – Observed photodegradation rate (k_{obs}), half-life time ($t_{1/2}$) in hours and in SSD for the adjustment to a pseudo-first order kinetic for the photodegradation of sertraline in the natural superficial waters.

Matrix	*n*	r^2	$k_{obs} \pm \sigma$ [h^{-1}]	$t_{1/2} \pm \sigma$ [h]	$t_{1/2} \pm \sigma$ [SSD]
RA	24	0.9725	0.032 ± 0.002	22 ± 1	5.7 ± 0.3
VR	18	0.9953	0.043 ± 0.001	16.1 ± 0.4	4.2 ± 0.1

Minor *et al.* (2006) reported the reductions in DOC concentrations in matrices of higher salinity during the irradiation of natural and artificial samples (the irradiation tests were carried out during 24h, with a lightbox at wavelength range of 295 to 365 nm). If the same phenomena occurred during this experiment, it could mean that, during the irradiation of SER.HCl in RA matrix (higher salinity than VR matrix), the DOM concentration (which would normally contribute to the indirect photodegradation of sertraline) would have been reduced during the irradiation. Thus, in that case, the observed lower photodegradation rate of the irradiation in RA matrix, in comparison to that of the irradiation with VR matrix, would be due to the lower salinity of the VR matrix.

3.4.3 Dilution of a WWTP's effluent matrix

The first collected sample (WWTP1) was subjected to single time irradiations and several dilutions of the matrix. This allowed for a more comprehensive study of the photodegradation mechanisms and, more importantly, enabled the study of the possibility of inhibition by organic matter and/or other constituents.

After 30h of irradiation, it is clear that the higher the fraction of WWTP1 sample present in the matrix, the lower the final concentration of SER and thus, the higher the photodegradation rate. This variation is linear up to 50% of WWTP1 (v/v) in the sample. Nevertheless, the difference between the irradiation with 50% and 90% of WWTP1 (v/v), is not as significant, indicating that this increase in the matrix concentration did not result in a noticeable increase in the photodegradation rate as described above. This fact might be an indicative that the photosensitizing effect observed for the diluted matrixes could be levelled out by phenomena that result in the inhibition of the photodegradation, such as light screening of the radiation for high concentrations of organic matter.

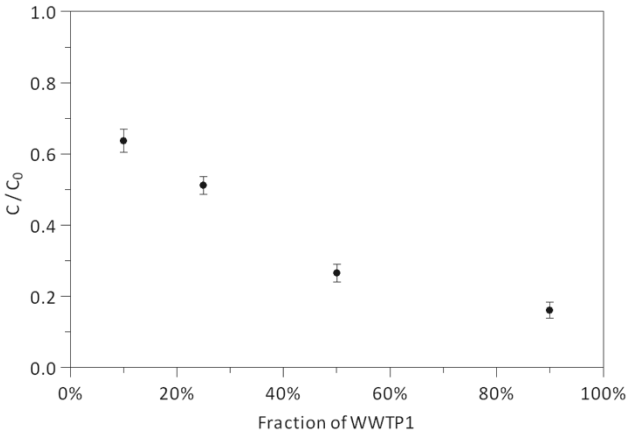

Figure 3.8 – Single time irradiations (30 h) of SER.HCl in the presence of different concentrations of a natural matrix (WWTP1). Each point (± standard deviation) represents the average of three replicates.

3.5 Influence factors on photodegradation of sertraline

3.5.1 Organic matter

As mentioned previously, single time irradiations of 30h were performed for different concentrations of the three humic substances (fulvic and humic acids and XAD-4 fraction), as these are all known to be active photosensitizing agents. Regardless, attending to the molecular differences between each of these fractions, the possibility of inhibition of photodegradation must be also considered, as this inhibition might be only noticeable for one of the substances, or for a range of concentrations of said substances.

When irradiating sertraline in the presence of different humic acid concentrations, there is a high increase in the photodegradation from 0 to 5 mg/L HA (Figure 3.9); however, for higher concentrations no further acceleration of the photodegradation processes was observed. In fact, the degradation observed for the highest concentrations of humic acids are inferior to those obtained for 5 mg/L, indicating that phenomena that lead to the decrease of photodegradation are occurring simultaneously to photosensitizing effects. The degradation in the presence of fulvic acids and XAD-4 fraction appear to have a similar behaviour, although less accentuated. There is a clear increase of the photodegradation rate by increasing the concentration of each substance. However, from a certain concentration on, the photodegradation percentage attained a plateau. This further sustains the likelihood of the occurrence of a filter effect caused by the high concentration of organic matter, where the radiation that could be absorbed by the pharmaceutical is, in turn, absorbed by the organic matter.

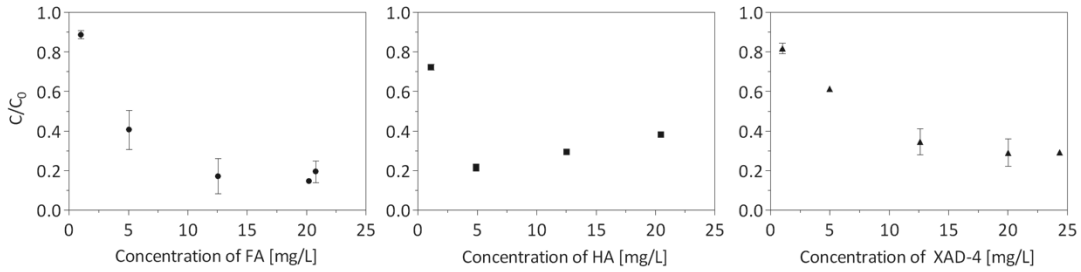

Figure 3.9 – Single time irradiations (30 h) of SER.HCl in the presence of different concentrations of humic substances (FA, HA and XAD-4). Each point (± standard deviation) represents the average of three replicates.

It is important to note that these are single irradiations and the behaviour of the kinetic behaviour of sertraline in the presence of these substances during the initial 30 hours of irradiation is unknown, requiring further kinetic studies, as shown below.

Despite all the differences in the structural composition between each of the humic substances, they all have at least one thing in common – carbon, which is here measured as dissolved organic carbon (DOC). By analysing all these irradiations in relation to their DOC content, it is possible to identify a relative trend. The increasing concentration of DOC seems to result in a greater photodegradation of sertraline, but it does not follow a linear pattern, it appears to almost become stagnant at a 0.3 C/C_0 level of degradation for concentrations higher than 6 mg/L of DOC.

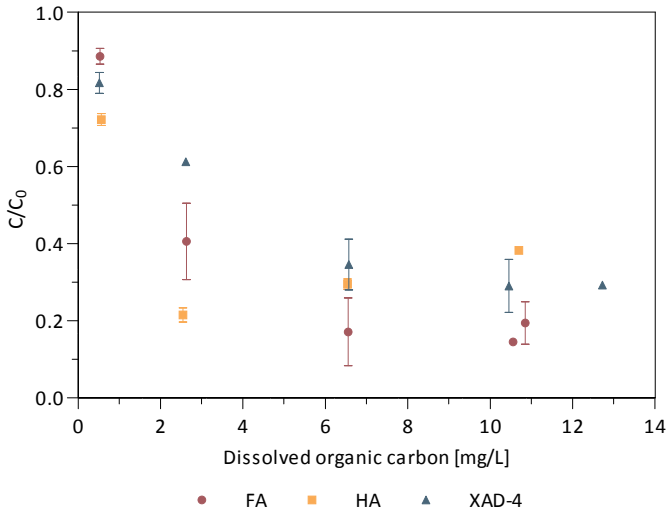

Figure 3.10 – Single time irradiations (30h) of SER.HCl in the presence of humic substances (FA, HA and XAD-4) in function of the dissolved organic carbon concentration in each irradiation. Each point (± standard deviation) represents the average of three replicates.

The kinetic study of the influence of each humic substance was carried out with the following concentrations: 20.1 mg/L FA, 12.6 mg/L HA and 20.0 mg/L XAD-4 (Figure 3.11).

The irradiation in the presence of humic acids, which had previously been shown to cause a filter effect at higher concentrations, resulted in a faster degradation rate, reaching the half-life at almost 12h, significantly lower than the other humic substances (Table 3.6).

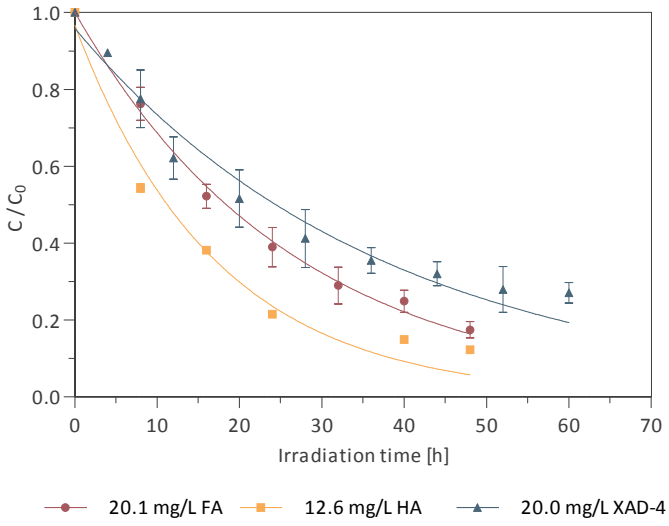

Figure 3.11 – Kinetic fitting to pseudo-first order model for the degradation of SER.HCl using synthetic matrices containing different humic substances (FA, HA and XAD-4). Each point (± standard deviation) represents the average of three replicates.

The irradiations of sertraline in the presence of FA and XAD-4 showed a low determination coefficient, not because of a poor fitting to the kinetic model, but because each irradiated tube appeared to have degraded at different rates, highly increasing the standard deviation of the average photodegradation for each irradiation time. This behaviour was not observed in the dark controls and did not occur during any other long irradiation, so it cannot be due to the irradiating apparatus (because the tube positions, irradiation intensity, and other parameters were constant throughout the study). Considering the standard deviations to each point of the kinetics with FA and XAD-4, the two kinetics do not appear to differ significantly.

Table 3.6 – Observed photodegradation rate (k_{obs}), half-life time ($t_{1/2}$) in hours and in SSD for the modelling to a pseudo-first order kinetic for the photodegradation of sertraline in synthetic matrices containing humic substances.

Matrix	*n*	r^2	$k_{obs} \pm \sigma$ [h^{-1}]	$t_{1/2} \pm \sigma$ [h]	$t_{1/2} \pm \sigma$ [SSD]
FA	21	0.9638	0.038 ± 0.002	18 ± 1	4.8 ± 0.3
HA	18	0.9766	0.059 ± 0.003	11.8 ± 0.6	4.2 ± 0.2
XAD-4	30	0.9421	0.027 ± 0.001	26 ± 1	6.9 ± 0.4

3.5.2 Ferric substances and Nitrates

The study of the influence of iron in the photodegradation of sertraline was performed in solutions of ultrapure water with a concentration of iron (in the form of Fe(III)) similar to the concentrations found in the natural samples (effluents and superficial waters). During the irradiation, there seemed to be a long delay in the degradation of sertraline (lasting 18 hours) followed by a sudden increase in degradation (see Figure 3.12).

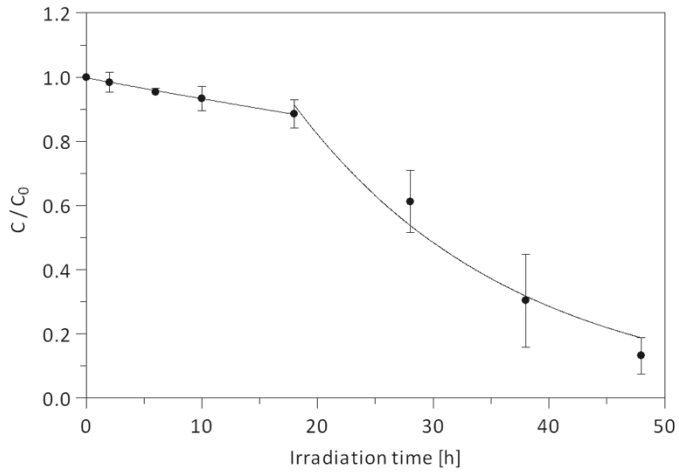

Figure 3.12 – Kinetic fitting to pseudo-first order model for the degradation of SER.HCl in the presence of Fe(III). Each point (± standard deviation) represents the average of three replicates.

This type of delay was also observed by Jakimska *et al.* (2014) during the irradiation of a treated water matrix spiked with sertraline and under natural solar light; however, the matrix composition was not disclosed and the irradiation conditions were different, thus not allowing a clear comparison between these two experiments. The authors attributed the phenomena to an autocatalytic reaction, triggered by the formation of a certain amount of a specific degradation product that allowed the photodegradation process to run consecutively.

The kinetic modelling was done for the two periods of irradiation – until the 18 h, and after the 18 h of irradiation (see Table 3.7). Since the first period of irradiation consisted of a slow irradiation, its adjustment was not considered in the calculation of the final half-life time; instead, the half-life time corresponded to the sum of the 18 hours of slow degradation to the half-life time calculated in the second photodegradation period, achieving a $t_{1/2}$ value of 31 ± 2 hours, equivalent to 8.3 ± 0.4 SSD.

Table 3.7 – Observed photodegradation rate (k_{obs}), half-life time ($t_{1/2}$) in hours and in SSD for the modelling to a pseudo-first order kinetic for the photodegradation of sertraline in 0.2 mg/L Fe(III).

Period	*n*	r^2	$k_{obs} \pm \sigma$ [h^{-1}]	$t_{1/2} \pm \sigma$ [h]	$t_{1/2} \pm \sigma$ [SSD]
0h to 18h	15	0.7341	0.007 ± 0.001	103 ± 17	27 ± 5
18h to 50h	12	0.9071	0.053 ± 0.007	13 ± 2	3.5 ± 0.4

One particularity observed during this irradiation is the fact that the first period of photodegradation resembles sertraline's kinetic under direct photodegradation (k_{obs} of 0.0062 h^{-1}). This suggests that during this period there was not an influence in the photodegradation from the ferric substances present in the solution.

The underlining factors that caused the delay and the subsequent fast photodegradation of sertraline are unknown. Ferrous substances (obtained after the oxidation of ferric substances) need to be in the presence of H_2O_2 in order to achieve its greatest photosensitizing effect, through the production of hydroxyl radicals and ions (HO• and OH^-), but it is also a process very dependant on the pH of the solution. Thus, there may have been variations to the pH of the solution caused by the irradiation which influenced the production of some of the photosensitizers.

The fact that this effect was not verified on the irradiations of sertraline in natural matrices (which have similar iron concentrations) may be due to the combined influence of other substances present in those particular matrices.

In addition to the kinetic study, the influence of ferric substances was studied in two other irradiations. Firstly, sertraline was mixed with a solution composed of ferric carboxylate species (which Chen *et al.* (2013) claim is a better representation of the species present in the environment) at the same concentration. Additionally, another solution of sertraline and solely ferric species was prepared, and both of these solutions underwent single time irradiations (30 hours). The influence of nitrates was studied through single time irradiations, by mixing sertraline with a solution containing NO_3^- (in the form of $NaNO_3$).

Table 3.8 – Single time irradiations (30h) of SER.HCl in the presence of ferric substances (Fe(III)) and carboxylate groups (oxalate) and nitrates.

Matrix	Matrix concentration	n	C/C_0 *
H_2O	-	3	0.81 ± 0.02
Fe(III)	0.6 mg/L Fe(III)	3	0.12 ± 0.02
Fe(III)-oxalate	0.6 mg/L Fe(III) and 13 mg/L C_2O_4	3	0.33 ± 0.02
NO_3^-	10 mg/L NO_3^-	3	0.28 ± 0.02

*after 30 hours of irradiation

The irradiations with ferric substances lead to unexpected concentrations of sertraline. In theory, the irradiation of SER.HCl with Fe(III)-oxalate should result in smaller concentrations than with Fe(III), mainly because the carboxylate group allows for the development of hydrogen peroxide that, in turn, reacts with Fe(II) (oxidated Fe(III) due to the irradiation) to provide the hydroxide and hydroxyl radical (explained in subchapter 1.4.3.2) for the indirect photodegradation of sertraline. However, the results show that a higher photodegradation was reached in the absence of oxalate, than in its presence.

Regardless of the differences between the two types of ferric synthetic matrices, it is very clear that the addition of Fe(III) influences the photodegradation of sertraline significantly, which increased the degradation of SER.HCl in almost 60% relatively to the photodegradation of sertraline in ultrapure water.

The irradiations of sertraline in the presence of NO_3^- resulted in a lower concentration, reaching 0.28 C/C_0 (Table 3.8) after the 30 hours of irradiation, which is similar to the concentration obtained with the single time irradiations with humic substances, in a concentration range of 6 to 13 mg/L DOC. The dark controls of this irradiation did not show signs of degradation, meaning that all the degradation that occurred to sertraline was due to the direct irradiation on sertraline and the nitrates, leading to the indirect photodegradation, possibly due to the hydroxide and hydroxyl radical formed after the photolysis of the nitrates (see subchapter 1.4.3.3).

3.5.3 Presence/Absence of oxygen

The influence of oxygen was studied through the irradiation of SER.HCl sparging the solutions with O_2 and N_2, including dark controls for both conditions. The experiment was carried out twice, with two samples for each condition (sparged with O_2 or N_2) and one control for each condition in the first experiment and two in the second experiment (totalling three replicates for each condition).

Neither the solutions sparged with O_2 nor with N_2 resulted in higher degradations compared to the not sparged solution (Figure 3.13). The solutions sparged with N_2 (*i.e.* in the absence of oxygen) seemed to inhibit the photodegradation of sertraline, as both the irradiated samples and the dark controls achieved C/C_0 (± standard deviation) values that are not significantly different.

The solution sparged with O_2 did not show degradation of the dark controls. Given the similarities between the degradations in the presence and absence of oxygen, it can be concluded that the presence of oxygen is not the most significant factor in the photodegradation of sertraline;

however, its presence may influence the mechanisms of formation of reactive species that promote the photodegradation rate of sertraline.

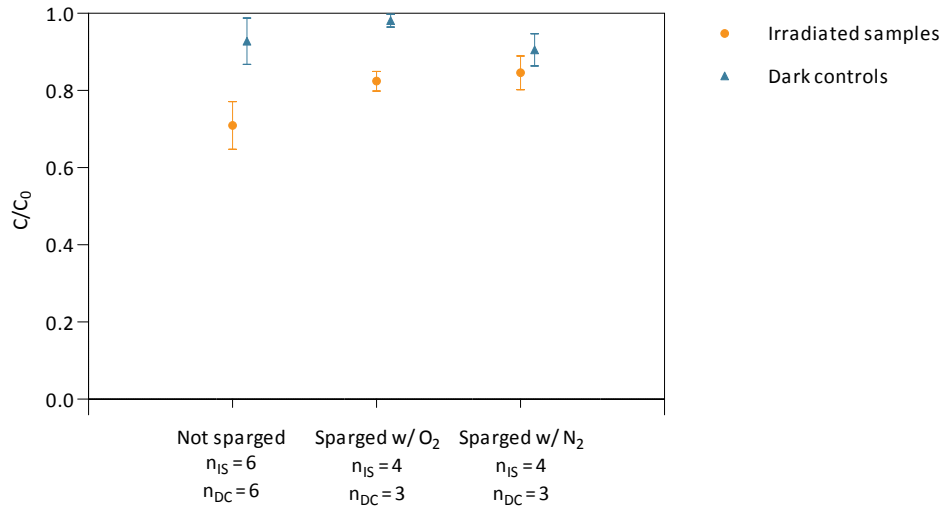

Figure 3.13 – Single time irradiations (30h) of SER.HCl not sparged and sparged with O_2 and N_2. Each point (± standard deviation) represents the average of n replicates (as presented on the axis of the graph).

3.6 Photosensitizing effect in natural and synthetic matrices

In order to evaluate the possible influence of each photosensitizer in the photodegradation of sertraline, the observed photodegradation rate constant was plotted as a function of the concentration of photosensitizer in each matrix (see Figure 3.14).

The effect of iron in the photodegradation matrix seemed to enhance the degradation of sertraline, *i.e.* for higher concentrations of iron, higher photodegradation observed rates were obtained. The presence of dissolved organic carbon seems to have an irregular influence. The irradiations in presence of humic substances showed how substances that had similar dissolved organic carbon content resulted in different degradation values of sertraline after 30 hours of irradiation. This shows that the type of molecules that constitute the organic matter have different behaviours during the irradiation. Thus, a matrix as complex as those collected from WWTPs and superficial water bodies may just as well have different organic carbon fractions, which could be the reason behind the varying photodegradation rates. The concentration of nitrates and nitrites does not seem to be a decisive factor in the photodegradation of sertraline. No kinetic studies were done to evaluate the photodegradation rate associated to the effect of solely nitrates and nitrites, thus the influence of these photosensitizers on the photodegradation of sertraline is inconclusive.

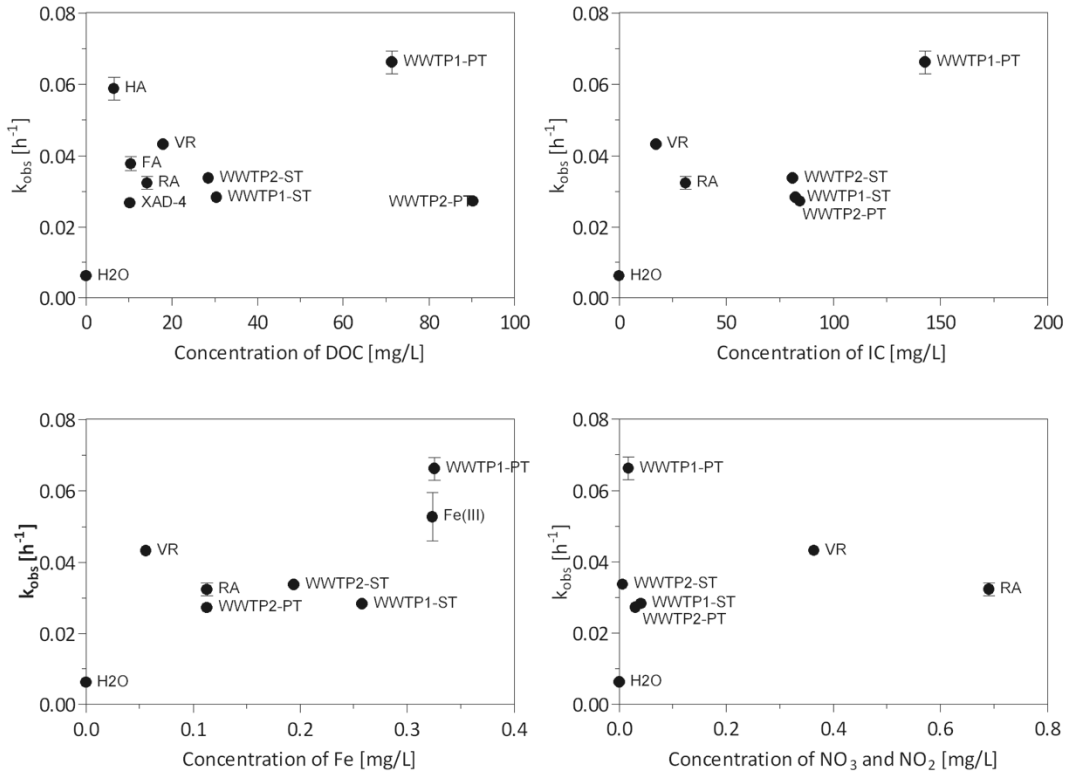

Figure 3.14 – Photodegradation rate constants in function of the concentrations of a) DOC, b) IC, c) Fe and d) NO3 and NO2.

The study of the most influencing factors of each matrix for the photodegradation of sertraline would require a thorough characterization of all the substances present in the matrices. Moreover, the study of the influence of each of those substances in controlled conditions (such as the of influences of iron and different concentrations of DOC explored in this study) and their combined effect (*e.g.*, a matrix composed of iron and DOC to verify if one inhibits the other) is required.

3.7 Detection of photoproducts by HPLC-UV

During the irradiations, aside from monitoring the concentration of sertraline, the chromatograms were also analysed for the formation (and evolution) of new peaks, that should be attributed to the generation of photoproducts.

The chromatograms of the irradiated samples, dark controls and matrix control were analysed and only the peaks detected in the irradiated samples, but not present in the dark controls and in the matrix control were considered as possible photoproducts.

The molecular structure and formula of the possible photoproducts are unknown and, within the HPLC-UV capabilities, they are impossible to identify but can be monitored by studying the variation of the peak areas along the photodegradation. As the UV detector was set to 210 nm, however, some of the photoproducts might not absorb at all (not detected) or have a better absorptivity at a different wavelength (low peak areas).

There is an evident variability in retention times of sertraline which is explained by the fact that each matrix has a different effect on the retention time of the analyte that may cause delays in the time the analyte remains in the stationary phase (C18). However, sertraline's retention time for each matrix was confirmed by the injection of the prepared stock solution for each irradiation.

The retention times of each photoproduct identified in the irradiation of sertraline in the various matrices are presented in Table 3.9. A value was attributed to each peak in order to simplify the identification of the peak during the analysis of its evolution, and does not imply that it is the same in each matrix. *E.g.*, the photoproduct identified by peak 3 in H_2O matrix does not imply that it is the same photoproduct identified as peak 3 in XAD-4 matrix.

Table 3.9 – Mean retention times ($t_{retention}$) and respective standard deviation (σ) for the peaks identified throughout the long irradiations for each matrix. *n* represents the number of occurrences of the peak during the irradiation. The retention times in bold represent the retention time of the main analyte, sertraline.

	$t_{retention} \pm \sigma$ [min] (*n*) per matrix										
Peak	**H_2O**	**FA**	**HA**	**XAD-4**	**Fe(III)**	**WWTP1-PT**	**WWTP1-ST**	**WWTP2-PT**	**WWTP2-ST**	**RA**	**VR**
1	3.28 ± 0.07 (18)	2.58 ± 0.07 (18)	2.72 ± 0.12 (15)	2.73 ± 0.13 (27)	2.87 ± 0.02 (18)	3.051 ± 0.006 (6)	4.84 ± 0.01 (21)	2.96 ± 0.08 (21)	3.80 ± 0.01 (18)	2.93 ± 0.15 (18)	2.847 ± 0.004 (12)
2	3.55 ± 0.09 (25)	3.04 ± 0.09 (18)	3.25 ± 0.17 (15)	3.20 ± 0.14 (27)	3.04 ± 0.08 (18)	3.21 ± 0.03 (12)	**5.30 ± 0.04 (21)**	3.54 ± 0.12 (21)	5.05 ± 0.11 (21)	3.22 ± 0.09 (18)	3.041 ± 0.007 (18)
3	3.80 ± 0.12 (26)	3.55 ± 0.13 (17)	4.93 ± 0.34 (14)	4.01 ± 0.38 (21)	3.43 ± 0.04 (17)	3.44 ± 0.03 (12)	9.74 ± 0.04 (12)	5.41 ± 0.21 (21)	**5.59 ± 0.19 (21)**	3.43 ± 0.10 (18)	3.227 ± 0.008 (18)
4	5.38 ± 0.48 (27)	4.61 ± 0.21 (16)	**5.40 ± 0.39 (15)**	**4.95 ± 0.25 (27)**	4.082 ± 0.025 (16)	3.92 ± 0.04 (12)	10.14 ± 0.04 (12)	**5.92 ± 0.27 (21)**		3.65 ± 0.09 (18)	3.63 ± 0.01 (18)
5	**5.88 ± 0.52 (27)**	**5.03 ± 0.21 (18)**			5.42 ± 0.02 (12)	5.80 ± 0.03 (6)		9.99 ± 0.05 (12)		4.03 ± 0.17 (15)	3.92 ± 0.02 (9)
6					**5.75 ± 0.14 (21)**	6.11 ± 0.07 (12)		10.38 ± 0.06 (12)		5.531 ± 0.008 (3)	5.55 ± 0.03 (18)
7					10.00 ± 0.02 (6)	**6.77 ± 0.18 (12)**				5.78 ± 0.03 (9)	**6.15 ± 0.09 (18)**
8					10.42 ± 0.02 (6)	9.97 ± 0.02 (6)				6.05 ± 0.42 (21)	9.98 ± 0.01 (9)
9						10.41 ± 0.02 (6)				**6.65 ± 0.60 (21)**	10.37 ± 0.01 (9)
10										10.01 ± 0.03 (12)	
11										10.34 ± 0.03 (12)	

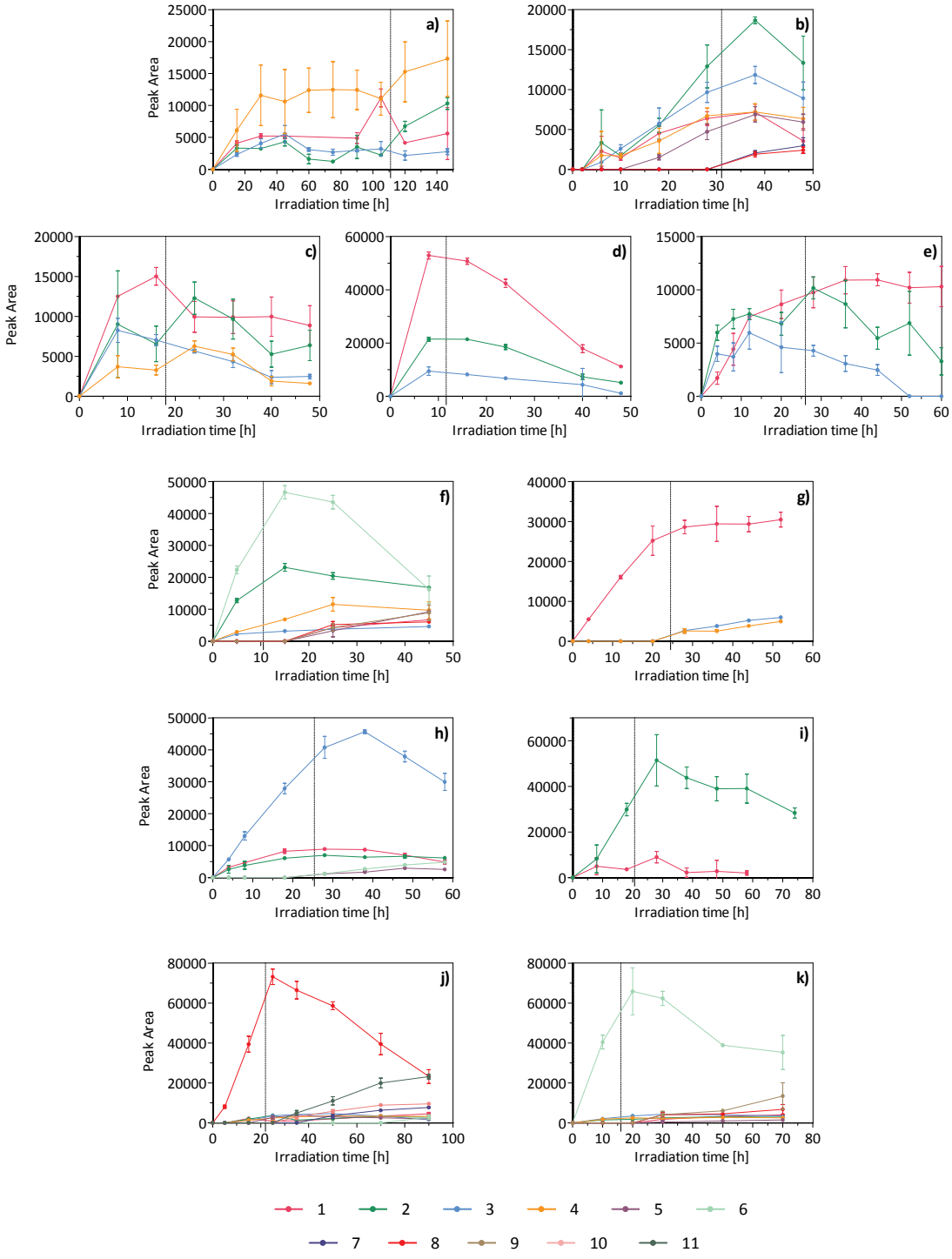

Figure 3.15 – Peak areas of the photoproducts detected by HPLC-UV during the irradiation of sertraline in matrices composed of a) ultrapure water, b) Fe(III), c) fulvic acids, d) humic acids, e) XAD-4, f) WWTP1-PT, g) WWTP1-ST, h) WWTP2-PT, i) WWTP2-ST, j) RA and k) VR. The dotted vertical lines correspond to the half-life time of sertraline. Each point (± standard deviation) represents the average of three replicates.

The direct photodegradation (irradiation in ultrapure water) of sertraline (Figure 3.15 a)) resulted in four photoproducts, all of which seem to be developed during the first 20 hours of irradiation and stabilize until approximately 100 hours of irradiation. At this point, two of the photoproducts increase their peak area, which coincides with the half-life time of sertraline in this matrix. One of the photoproducts displays a sudden peak at 100 hours but resumes its steady peak area at the next sampling time.

The irradiation of sertraline in the presence of ferric substances (Figure 3.15 b)) resulted in a very similar behaviour among all the detected photoproducts. Despite the unusual behaviour of sertraline during this irradiation, with a delay of 18 hours followed by a quick degradation, its photoproducts display an interesting evolution, with its steady increase over time. The formation of photoproducts would require a degradation of sertraline molecules, and therefore, an indication of its concentration decline and an increase of the photoproducts' concentration. As mentioned previously, the concentration of sertraline during its irradiation with Fe(III) resembles its behaviour during the direct photodegradation test, until approximately 20 hours of irradiation. During these hours the photodegradation products steadily increase in both of the matrices; the local peak identified at 6 hours in the ferric matrix was not detected in the water matrix as the first sampling was done at 15 hours, but this does not provide evidence that it did not occur as well during the direct photodegradation test.

Out of the three irradiations with humic substances, the irradiation with HA resulted in the clearest evolution of photoproducts (Figure 3.15 d)), with a very clear peak at the first sampling moment (8 h) and a slow decrease during the rest of the irradiation. The photoproducts derived of the irradiations with FA (Figure 3.15 c)) and XAD-4 (Figure 3.15 e)) had high deviations among samples but, overall, both show an increase in peak areas of the photoproducts at the beginning of the irradiations. Some photoproducts increase in peak area and remain throughout the irradiation, *e.g.* the first peaks in each of the matrices.; while others increase and show a clear decrease, *e.g.* peak 3 on FA and peak 3 on XAD-4 (the latter completely disappeared by the end of the study).

During the irradiations in wastewater matrices (Figure 3.15 f), g), h) and i)), it is very clear that the irradiations carried in wastewater from primary treatments result in a considerably higher number of detected photoproducts, in contrast to the small number detected in wastewater from secondary treatments. This might be due to two factors, one related to the method of detection and the other to the differences between matrices, or both. Wastewater from primary treatment, even after the filtrations, had a significantly darker colour (yellow tone) and unpleasant odour than

that of the wastewater from secondary treatment, which is natural, given the main objective of secondary treatments in WWTPs. These differences in the physical properties of the wastewater matrices suggest differences in composition which may interfere in the photodegradation processes responsible for the removal of sertraline. Thus, this can be reflected in the photoproducts from these reactions. The method of detection also cannot be ignored, as the nature of the matrices might interfere with the detection of some photoproducts. Even though there were no observed interferences in the detection and quantification of sertraline in any of the matrices, this does not rule out the fact that other substances may pass undetected through the HPLC-UV.

The photoproducts derived from the degradation of sertraline in wastewater matrices show that all photoproducts reached a maximum peak after the half-life time of sertraline. Some of these photoproducts show a decrease in peak area until the end of the tests (peaks 2 and 6 in WWTP1-PT, peaks 1 and 3 in WWTP2-PT and peak 2 in WWTP2-ST), but some, particularly peak 1 in WWTP1-ST and some of the photoproducts of lower peak areas do not show signs of decrease during the irradiation period.

Likewise, the behaviour registered in the photoproducts in the irradiations of superficial water (Figure 3.15 j) and k)) also show the highest peak areas after the half-life time of sertraline in each matrix.

Two particular photoproducts were purposely not mentioned above, as they all appear to show the same behaviour. These photoproducts are as follows: peaks 7-8 in Fe(III), peaks 8-9 in WWTP1-PT, peaks 3-4 in WWTP1-ST, peaks 5-6 in WWTP2-PT, peaks 10-11 in RA and peaks 8-9 in VR. The photoproducts appear only far into the degradation of sertraline, suggesting that they might result from the degradation of other photoproducts already existing in the solution. These two peaks show two common factors: they either occur approximately at the same time of the half-life time of sertraline (*e.g.*, in matrices Fe(III) and WWTP2-ST), near the maximum peak area of the photoproduct with highest peak areas (*e.g.*, VR), or a combination of both (*e.g.*, WWTP1-PT, WWTP1-ST and RA).

3.8 Identification of photoproducts by mass spectrometry

Sertraline prepared in ultrapure water, at a concentration of 5 mg/L SER.HCl, was irradiated for 100 hours, including a dark control. The mass spectra of the irradiated sample, the dark control and the standard solution were obtained according to the procedure presented in subchapter 2.4.2.

The selection of photoproducts to analyse by ESI-MS^2 was done by comparing the ESI-MS spectra of the standard solution the irradiated sample and the dark control (see Figure 3.16). The peaks that were exclusively observed in the irradiated sample were considered as possible photodegradation products. Through the analysis of the fragmentation of those peaks and the isotopic patterns in the MS spectrum (which allows to conclude about the presence, or not, of chlorine), to each photoproduct was proposed a molecular formula and structure (see Figure 3.17 and Table 3.10).

The ESI-MS^2 spectra of photoproducts A, B, D, E and F all display fragmentation peaks in accordance with the loss of oxygen groups, either in the form of an H_2O group (losses of 18 Da) or a CO group (losses of 28 Da), indicating the presence of these moieties in the structure of the photoproduct. Photoproducts A and B only display losses of H_2O groups or alcohol chains, and given their low molecular weight, it was considered that these photoproducts corresponded to the loss of the amine and di-chlorine benzene ring functional groups relatively to the initial molecule of sertraline. The isotopic pattern of photoproducts C, D, E and F in the ESI-MS spectrum are consistent with the presence of Cl_2, and given that the molecular weight is close to that of sertraline, it was considered that there were only losses of the amine group after the irradiation (photoproducts C and D), and the bonding of oxygen groups to the molecule (photoproducts D, E and F). The fragmentations which support the structures proposed for each photoproduct are detailed in Table 3.10.

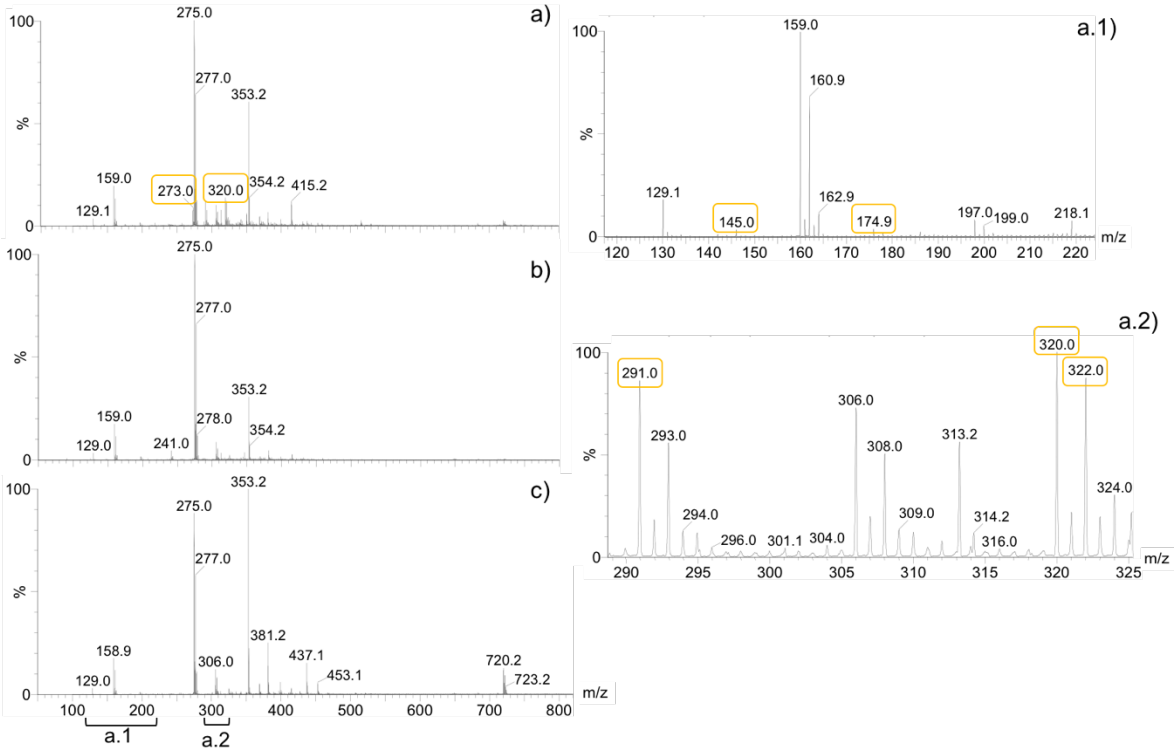

Figure 3.16 – ESI-MS spectra of a) irradiated sample (100 h), b) dark control and c) standard solution of sertraline; a.1) Detailed view of ESI-MS spectrum of irradiated sample from 120 to 220 *m/z*; a.2) Detailed view of ESI-MS spectrum of irradiated sample from 290 to 325 *m/z*. The marked peaks (yellow squares) correspond to possible photodegradation products.

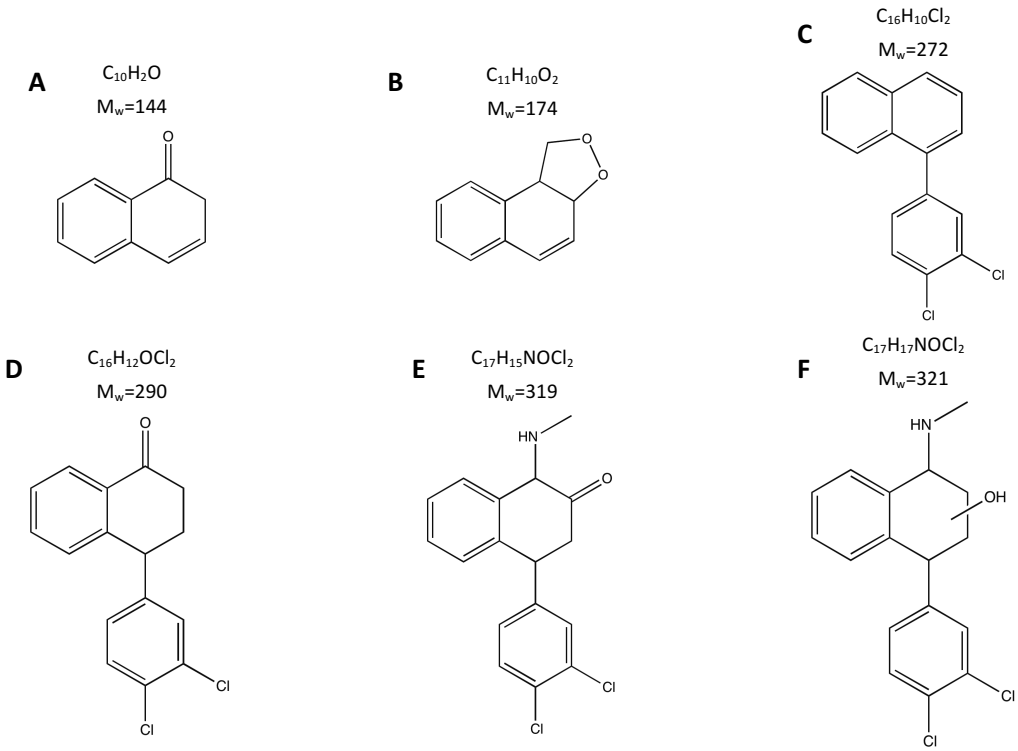

Figure 3.17 – Proposed molecular formula and structure for the identified photoproducts of sertraline and corresponding molecular weight.

Table 3.10 – Fragment ions detected in ESI-MS^2 of each of the selected photoproducts, identified by ESI(+)MS, collision energy used for the fragmentation and proposed molecular formulas.

Proposed molecular formula	M_w	ESI(+)MS *m/z* *	Collision energy [eV]	ESI(+)MS^2 *m/z* (relative abundance %, loss)
Sertraline $C_{17}H_{17}NCl_2$	305	306	15	275 (100%, -H_3CNH_2); 159 (12%, -$C_6H_5Cl_2$); 129 (2%, -$C_7H_9NCl_2$)
A: $C_{10}H_8O$	144	145	10	117 (31%, -CO)
B: $C_{11}H_{10}O_2$	174	175	10	157 (15%, -H_2O); 143 (47%, -H_3COH); 129 (39%, -C_2H_5OH)
C: $C_{16}H_{10}Cl_2$	272	273	25	238 (100%, -Cl); 203 (9%, -Cl_2)
D: $C_{16}H_{12}OCl_2$	290	291	25	256 (14%, -Cl); 221 (2%, -Cl_2); 175 (25%, -C_2H_5OH)
E: $C_{17}H_{15}NOCl_2$	319	320	35	289 (12%, -H_3CNH_2); 261 (18%, -H_3CNH_2 and -CO); 254 (100%, -H_3CNH_2 and -Cl); 226 (48%, -H_3CNH_2 , -CO and -Cl); 219 (18%, -H_3CNH_2 and -Cl_2); 201 (16%, -H_3CNH_2 , -H_2O and -Cl_2); 191 (39%, -H_3CNH_2 , -CO and -Cl_2);
F: $C_{17}H_{17}NOCl_2$	321	322	35	307 (4%, -CH_3); 291 (10%, -H_3CNH_2); 273 (8%, -H_3CNH_2 and -H_2O); 256 (17%, -H_3CNH_2 and -Cl); 238 (100%, -H_3CNH_2 , -H_2O and -Cl); 228 (15%, -H_3CNH_2 , -CO and -Cl); 203 (10%, -H_3CNH_2 , -H_2O and -Cl_2)

* m/z values of ESI(+)MS correspond to $[M+H]^+$.

The molecules corresponding to the $[M+H]^+$ values of 273, 291 and 322 (photoproducts C, D and F) were also identified in a photodegradation study of sertraline (Jakimska *et al.* 2014). Although the fragment ions of the present study did not match exactly those obtained by Jakimska *et al.* (2014), the molecular structures here proposed were consistent with the molecular structures proposed by the referred paper.

Chapter 4 Conclusions and final remarks

The direct photodegradation of sertraline (SER) resulted in a half-life time of 111 hours (under simulated solar light), which is a considerably long period of time for a substance to be degraded considering its corresponding degradation time in Summer Sunny Days (SSD), 29 days. Due to its low absorptivity, it is possible that the photodegradation was either a result of the influence of oxygen naturally present in the solution or to a higher quantum yield of sertraline.

The measured half-life was converted to SSD with a conversion factor that considers that all the irradiation days are performed with a clear sky and light intensity typical of a summer day. The value of 29 SSD obtained for direct photodegradation differs significantly from the two results presented in the literature of 127.5 days (with a delay of 120 days) at pH 3 and 9.7 days at pH 10, both obtained with natural solar light (Jakimska *et al.* 2014). These differences could be due to two factors: firstly, the referred paper was performed in highly acidic and highly basic mediums, and the effect of pH was shown to be significant in the photodegradation of sertraline, whereas the present study was conducted without pH adjustments (the pH of ultrapure water is usually in the value range of 5 to 6); secondly, the half-life obtained in this study was estimated with controlled irradiation, without the influence of solar light fluctuations and the final value in SSD assumes that all the irradiation days would be under a clear sky. The study done by Jakimska *et al.* (2014) is presumed to have been conducted in Poland, which does not differ significantly in latitude from Italy (the original location from which the irradiation time conversion factor was estimated (Vione *et al.* 2006; Minero *et al.* 2007)), but the months during which the irradiation took place were not disclosed, neither the intensity of solar light and respective fluctuations (due to the weather conditions).

The study of the indirect photodegradation of sertraline was carried out by the irradiation of sertraline solutions with photosensitizers like dissolved organic carbon (DOC) (in the form of humic substances), iron or nitrates. The influence of the concentration of DOC was verified by single time 30 hour irradiations with different concentrations of photosensitizers; the photodegradation rate of sertraline in the presence of photosensitizers was performed for a single concentration of humic substances and iron.

The influence of organic matter was investigated through the irradiation of sertraline in the presence of three types of humic substances, known for their accurate representation of natural organic matter found in the environment. From these studies, it was concluded that the nature of the organic matter (*i.e.*, its composition at a molecular level) had differing effects on the degradation of sertraline. Humic acids affected the degradation sertraline faster than the other two

substances at a lower concentration of organic carbon (6.5 mg/L DOC), enabling sertraline to reach its half-life at 12h of irradiation, whereas fulvic acids and XAD-4 fraction, both at a concentration of approximately 10 mg/L DOC, lead to a half-life of 18h and 26h, respectively. This suggests that the nature of the organic carbon plays a fundamental role in the photodegradation of sertraline. Humic acids are known to be more hydrophobic than fulvic acids or the XAD-4 fraction and this property makes them more prone to adsorbing onto the surface of other molecules present in the aqueous solution. Thus, its hydrophobicity allow humic acids to bind to sertraline, forming humic-sertraline complexes in the aqueous phase (Klavins & Serzane 2000).

It was also observed that concentrations higher than 5 mg/L HA do not result in a higher degradation of sertraline. On the contrary, higher concentrations seemed to inhibit the full photosensitizing potential of HA, similar to a light screening effect.

Fulvic acids and the XAD-4 fraction exhibited similar behaviours regarding the concentration of organic matter and photodegradation percentage after 30 hours of irradiation: from 0 to 13 mg/L of either substance, the degradation of sertraline increased, but adding higher concentrations caused neither higher nor lower photodegradation percentages. Thus, of the three humic substances, humic acids displayed a higher potential for photon filtering effect at higher concentrations. Nonetheless, sertraline has a such a slow degradation rate and low absorptivity in the visible wavelength range that the presence of humic acids enhances the degradation of sertraline greatly, albeit its lower photosensitizing potential at higher concentrations.

The irradiations carried out in the presence of ferric substances showed a clear photosensitizing effect. The single-point irradiations were conducted with iron concentrations superior to those found in the natural matrices and reached photodegradation percentages in the range of 77%. However, the kinetic study that was done with a lower concentration, to mimic those found in the natural matrices, revealed a long delay in the photodegradation followed by a sudden degradation of sertraline. This effect was also observed by Jakimska *et al.* (2014) during the irradiation of sertraline in treated water, methanol and ultrapure water at pH 3.

The fact that the study identified this delay for lower levels of pH indicates that there is a possibility that the irradiation with Fe(III) may have suffered pH changes during the irradiation. However, lower pH levels have been shown to improve the degradation of organic compounds in the presence of Fe(III) (Machulek *et al.* 2012). Thus, further studies should be developed, with a steady control of the solution's pH and at different concentrations of ferric substances in order to verify the cause of the delay.

Nitrates and nitrites enhanced the photodegradation of sertraline, having achieved, after 30h of irradiation, concentrations similar to those obtained with over 6.5 mg/L DOC. This shows that the radical HO• formed due to the photolysis of NO_3, and also formed after the photolysis of humic substances, may play an important photosensitizing role in the degradation process of sertraline, but, to confirm this possibility, the irradiations with these photosensitizers should be repeated with the addition of a quencher of HO• radicals.

It was concluded that the presence of oxygen in the solution is not a factor or great influence in the direct photodegradation of sertraline, but its presence may contribute to the formation of reactive species that have been shown to enhance the photodegradation of sertraline through indirect photodegradation processes.

The photodegradation of sertraline in natural matrices was greatly enhanced in comparison to the results obtained for its direct photodegradation (irradiations in ultrapure water): half-life time of sertraline in natural matrices ranged from 10.5 h (in WWTP1-PT) to 25.4 h (in WWTP2-PT), *versus* the 111 h in ultrapure water.

A direct correlation between the type of wastewater (from primary or secondary treatment) and the photodegradation rate was not possible to achieve; irradiations of sertraline in wastewater from primary treatment were faster in samples from WWTP1 but slower in samples from WWTP2. Samples from primary treatment are considerably richer in organic matter and, because of this, a light screening effect was expected. However, given the significantly different observed photodegradation rates, there must have been at least one factor that enhanced the degradation in WWTP1-PT, but was not identified in this study.

Considering the fact that there were several more photoproducts detected during the irradiations with wastewater from primary treatments than from secondary treatment, it seems that there were a larger array of reactions occurring in the former than the latter. This is justified by the fact that raw wastewater is usually very complex in its composition and the primary treatments only remove most of the surface oils and the major part of grit and particulate matter. Hence, there is not an important influence of this treatment on the dissolved phase (which is essentially what was used in these experiments, due to the filtrations). Wastewater from secondary treatment, however, has been subjected to biological treatments that break down the micronutrients and organic carbon as well as other contaminants, rendering it to a less complex sample at a molecular level. Thus, sertraline in wastewater from secondary treatments is not subject to the presence of as many compounds, resulting in less photoproducts.

Taking into account the fact that the WWTPs from where the samples were collected have a hydraulic retention time of 24 hours, it is unlikely that sertraline would be degraded solely by the influence of the photosensitizing agents already present in the matrix (albeit in unknown and varying concentrations). However, factors like biodegradation would need to be studied, in order to develop proper procedures for the efficient removal of the contaminant from these effluents.

Researches presented earlier in this study have shown that the consumption of sertraline is considerably high in Portugal. However, the detected concentrations in wastewaters from different sites, including those where higher percentages of pharmaceutical compounds would be expected (*e.g.* hospital wastewater), were particularly low. From this study, it can be concluded that sertraline is persistent in the environment and is difficult to degrade by natural factors (such as photosensitizers naturally present in the waters) and the daily access to solar irradiation. The pharmaceutical itself is extensively metabolized in the human body into N-desmethyl-sertraline (a much less potent metabolite of sertraline). The low detected concentrations in aqueous matrices could be due to the high *log* K_{OW} and *log* K_{OC} values presented in the literature. Sertraline has a strong affinity to bonding the solid phase such as the sediments and soil at the bottom and sides of rivers, the sludge from WWTP processes and even particles present in the aquatic environments. The sample collection process altogether does not account for these adsorptions, as the procedure requires the filtration of the samples after collection, followed by the proper conditioning treatments.

A total of six photodegradation products, formed by direct photodegradation of sertraline, were identified by mass spectrometry. Three of these photoproducts were also identified by Jakimska *et al.* (2014), further sustaining the formation of these particular substances during the photodegradation of sertraline.

Finally, further studies should be developed on the identification of the mechanistic photodegradation of sertraline in natural matrices and in the presence of each relevant photosensitizer. Thus, an assessment of potential application of the photodegradation process under natural sunlight in a WWTP should be developed. However, the fate of the added photosensitizers is also a sensitive matter, as some could lead to even more harm towards aquatic organisms. Additionally, the potential toxicity of the products derived from the photodegradation of sertraline under UV/Vis radiation should be a subject of future studies.

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